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ORIGINAL RESEARCH

Combined Detection of Tumor Stem Cell Markers CD133 and OCT4 in Early Non-Small Cell Lung Cancer Screening and Prognostic Evaluation

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Objective: To investigate the expression of cancer stem cell (CSC) markers CD133 and OCT4 in early-stage non-small cell lung cancer (NSCLC), evaluate their diagnostic value in early screening, and analyze their prognostic significance.

Methods: A retrospective study was conducted on 80 patients with early-stage NSCLC (stages I–IIA) and 40 healthy controls from January 2021 to December 2023. Expression levels of CD133 and OCT4 were assessed by immunohistochemistry (IHC) and quantitative real-time PCR (qRT-PCR). Clinicopathological data were analyzed, and all patients were followed for 24 months to assess overall survival (OS). Diagnostic efficacy was evaluated by ROC analysis, and prognostic factors were identified by Cox regression.

Results: CD133 and OCT4 were significantly upregulated in NSCLC tissues compared with adjacent normal tissues and healthy controls ($P<0.001$). High expression correlated with poor differentiation, larger tumor size (≥3 cm), lymph node metastasis, and stage IB–IIA ($P<0.05$). ROC analysis showed AUCs of 0.809 for CD133, 0.796 for OCT4, and 0.893 for their combination, with combined sensitivity of 88.7% and specificity of 82.5%. Patients with high expression of both markers had markedly reduced 2-year OS compared with low-expression cases ($P<0.01$). Multivariate Cox regression identified high CD133 expression (HR=2.45, 95% CI: 1.38–4.36, P=0.003), high OCT4 expression (HR=2.17, 95% CI: 1.22–3.86, P=0.007), poor differentiation (HR=1.91, P=0.021), tumor size ≥3 cm (HR=1.84, P=0.039), lymph node metastasis (HR=2.08, P=0.020), and stage IB–IIA (HR=2.22, P=0.016) as independent prognostic risk factors.

Conclusion: CD133 and OCT4 are overexpressed in early-stage NSCLC and are associated with aggressive disease and poor prognosis. Combined detection provides superior diagnostic accuracy (AUC=0.893) compared to single markers and may serve as a valuable biomarker panel for early screening and risk stratification. These markers also have potential utility in guiding individualized treatment strategies.

Keywords: NSCLC, CD133, OCT4, cancer stem cells, early screening, prognostic evaluation

Introduction

Non-small cell lung cancer (NSCLC) is the most common histological subtype of lung cancer, accounting for more than 85% of all lung cancer cases.[1] Due to its insidious early symptoms and rapid progression, most patients are already at an advanced stage at diagnosis, missing the optimal treatment window and resulting in a persistently low overall five-year survival rate.[2] Although imaging techniques and liquid biopsy methods have improved in recent years, early screening of NSCLC still faces limitations such as low sensitivity and insufficient specificity.[3] Therefore, identifying efficient, stable, and reproducible biological markers to assist in early detection and risk stratification has become a key focus in current lung cancer research.

Cancer stem cells (CSCs) are a subset of tumor cells with self-renewal, infinite proliferation, and differentiation capabilities and are believed to be core drivers of tumor initiation, progression, drug resistance, and metastasis.[4,5] Among

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them, CD133, a pentaspan transmembrane glycoprotein, is widely expressed in the stem cell population of various tumor types,[6,7] and is one of the most extensively studied CSC markers. Previous studies[8] have shown that CD133-positive cells in NSCLC tissues exhibit strong proliferative and migratory abilities and are closely associated with poor tumor biological behavior. OCT4, an essential transcription factor for maintaining pluripotency in embryonic stem cells, has been found to be aberrantly expressed in a variety of malignant tumors, particularly in solid tumors such as lung cancer,[9] gastric cancer,[10] and esophageal cancer,[11] where its high expression is strongly correlated with poor differentiation, high metastatic potential, and poor prognosis. OCT4 is considered a key regulator in maintaining CSC stemness, and its upregulation often indicates enhanced self-renewal and invasiveness of tumor cells.[12]

At present, studies on CD133 and OCT4 in NSCLC mostly focus on patients with advanced stages, while their expression characteristics in early-stage NSCLC, the diagnostic value of combined detection for early screening, and their prognostic implications remain underexplored. Therefore, this study aims to investigate the expression levels of CD133 and OCT4 in tumor tissues from patients with early-stage NSCLC, analyze their correlation with clinicopathological parameters, and further evaluate their sensitivity and specificity—individually and in combination—for early screening. Additionally, through follow-up analysis, the study seeks to explore the prognostic significance of these markers, thereby providing theoretical and biomarker-based support for the early diagnosis, stratified treatment, and prognostic assessment of NSCLC.

Materials and Methods

Study Subjects

This study was a retrospective case-control study. The study population included patients diagnosed with early-stage NSCLC who were treated with surgery in the Department of Thoracic Surgery and Respiratory Medicine of our hospital from January 2021 to December 2023. All cases were pathologically confirmed by at least two associate chief pathologists or above through postoperative histopathological examination. Tumor clinical pathological staging was classified as stage I to IIA according to the 9th edition of the TNM classification system by the Union for International Cancer Control (UICC).[13] A total of 80 patients with early NSCLC were enrolled. The control group consisted of 40 healthy volunteers who underwent annual physical examinations at the hospital's health examination center during the same period and were confirmed to have no pulmonary diseases. Sex and age were matched with those of the NSCLC group, and the differences were not statistically significant ($P>0.05$). All controls had no family history of tumors, no history of chronic lung disease, and no significant abnormalities in chest CT, pulmonary function tests, or laboratory tests. Inclusion criteria: (1) Aged between 18 and 80 years, no gender restriction; (2) Pathologically diagnosed with primary early-stage NSCLC (stage I–IIA); (3) Underwent surgical treatment for the first time, and did not receive radiotherapy, chemotherapy, targeted therapy, or immunotherapy before surgery; (4) Complete surgical resection with sufficient qualified tumor tissue and adjacent normal lung tissue available for testing; (5) Complete clinical and follow-up data; (6) The patient and their family were fully informed about the study and signed informed consent. Exclusion criteria: (1) Coexisting or previously diagnosed with other malignancies; (2) Definite history of immune system disorders, hematologic diseases, or autoimmune diseases; (3) Distant metastases (stage M1) or intraoperative discovery of advanced stage disease; (4) Incomplete preservation of tumor tissue samples or severe RNA degradation; (5) Lost to follow-up or follow-up discontinued; (6) Severe comorbidities (eg, advanced liver or kidney dysfunction) that could affect prognostic evaluation.

Sample Size Basis and Ethical Approval

The sample size was determined based on expected expression differences of CD133 and OCT4 in NSCLC patients reported in previous literature (moderate difference, effect size ≈ 0.6). Calculations were performed using G*Power software. Under $\alpha=0.05$ and power=0.8, at least 70 NSCLC patients and 35 controls were required. To control for potential sample loss and ensure analysis stability, the final sample included 80 patients and 40 controls. This study was approved by the Ethics Committee of Changzhou First People's Hospital (Ethics Approval No.: NKFZ2507). Throughout

the research process, the Declaration of Helsinki and relevant ethical codes were strictly followed to ensure subject privacy and data security.

Clinical Data Collection

Basic and clinical information of all enrolled early NSCLC patients was extracted and verified by at least two full-time research staff members via electronic medical record systems, pathology systems, and surgical records, and cross-checked by a third person to ensure accuracy and consistency. Data collected included: sex; age; smoking history; presence of comorbidities (such as hypertension, diabetes, COPD); tumor histological type (eg, adenocarcinoma, squamous cell carcinoma, large cell carcinoma); tumor differentiation (moderate-to-high vs poor differentiation); tumor diameter; TNM stage (based on UICC 9th edition); lymph node metastasis status; presence of vascular or pleural invasion; and postoperative adjuvant therapy (chemotherapy, targeted therapy, immunotherapy).

Immunohistochemistry (IHC) Detection

IHC staining was performed using the streptavidin-biotin complex (SABC) method on conventional paraffin-embedded tissue sections, strictly following a standardized procedure: (1) Tissue Section Processing: Tumor and adjacent non-tumor tissue paraffin blocks from each case were selected. Sections were cut at 4 μm thickness, mounted on glass slides, and baked at 60°C for 2 hours. Deparaffinization was done with xylene (2×10 minutes), followed by gradient rehydration using 100%, 95%, 85%, and 75% ethanol (5 minutes each). After rinsing with tap water, antigen retrieval was performed using 0.01 mol/L citrate buffer (pH 6.0) heated in a microwave at medium-high power for 10 minutes, then naturally cooled to room temperature. Endogenous peroxidase activity was blocked by incubating with 3% hydrogen peroxide for 10 minutes at room temperature, followed by PBS washing (3×). (2) Antibody Incubation and Staining: Slides were incubated with normal goat serum blocking solution for 20 minutes at room temperature to block non-specific binding. Then, the primary antibodies were added: anti-CD133 polyclonal antibody (Abcam, Cat# ab19898, dilution 1:200); anti-OCT4 monoclonal antibody (Abcam, Cat# ab19857, dilution 1:150). Slides were incubated in a humid chamber at 4°C overnight. The next day, PBS was used for three washes (5 minutes each). HRP-conjugated secondary antibody was applied and incubated at room temperature for 30 minutes. DAB was used for color development (3–8 minutes), monitored under a microscope for optimal effect. Hematoxylin counterstaining was performed for 30seconds, followed by dehydration, clearing, and mounting. (3) Interpretation of Staining Results: Two experienced pathologists independently evaluated the stained sections in a double-blinded manner. If disagreement occurred, a third pathologist participated in discussion until consensus was reached. Cellular localization: CD133 was primarily expressed on the cell membrane; OCT4 was mainly localized in the nucleus. Scoring was performed using a semi-quantitative method, with total score = staining intensity × percentage of positive cells, ranging from 0 to 9. A total score ≥6 was defined as "high expression"; <6 was defined as "low expression". Details are shown in Table 1 below.

Quantitative Real-Time PCR (qRT-PCR)

Used to detect mRNA expression levels of CD133 and OCT4. The experimental procedure was as follows: (1) RNA Extraction: Total RNA from tumor and adjacent tissues was extracted using TRIzol reagent (Invitrogen, USA). RNase-free materials were strictly used throughout to avoid contamination. RNA concentration and purity were measured with a NanoDrop 2000 (Thermo Fisher), requiring A260/A280 between 1.8–2.1. Sample integrity was confirmed via 1% agarose gel electrophoresis, showing clear bands without degradation. (2) cDNA Synthesis: According to the instructions

Table 1 Semi-Quantitative Scoring Method

Staining Intensity Score	Positive Cell Percentage Score
No color (0)	<5% (0)
Light yellow (1)	5–25% (1)
Brown-yellow (2)	26–50% (2)
Dark brown (3)	>50% (3)

Table 2 Primer Sequences

Gene	Forward Primer (5′-3′)	Reverse Primer (5′-3′)
CD133	AGTGGCATTGACCGTTCTTG	GATCTTCTCCACCAACGCCT
OCT4	GAGGAAGCTGACAACAATGAGA	TTCTGGCGCCGGTTACAGAAC
β-actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT

of the reverse transcription kit (Takara, PrimeScript RT reagent Kit), 1 μg of total RNA was reverse transcribed into cDNA. The reaction volume was 20 μL, with conditions: 37°C for 15 min, 85°C for 5s. The synthesized cDNA was stored at −20°C for later use. (3) qPCR Amplification: Performed using SYBR Green PCR Master Mix (Takara) in a 20 μL system, including: SYBR Premix Ex Taq II: 10 μL; forward/reverse primers: 0.4 μL each (concentration: 10 μmol/L); cDNA template: 2 μL; ROX reference dye: 0.4 μL; ddH_2O to make up 20 μL. Amplification conditions: pre-denaturation at 95°C for 30s; denaturation at 95°C for 5s; annealing/extension at 60°C for 30s, for 40 cycles. (4) Primers: Synthesized by Sangon Biotech. The 2−ΔΔCt method was used to calculate relative expression levels. Each sample was tested in triplicate, and the average was used. β-actin served as the internal control gene for normalization. Negative controls were included in all assays to ensure amplification specificity and exclude contamination. Primer sequences are shown in Table 2.

Follow-Up and Prognostic Evaluation

Postoperative follow-up was conducted for 24 months via outpatient visits and telephone calls. Follow-up content included recurrence status, time of distant metastasis, and overall survival (OS). OS was defined as the time from surgery to death or the last follow-up.

Statistical Analysis

Statistical analyses were performed using SPSS 26.0, and charts were drawn with GraphPad Prism 9.0. Continuous variables following a normal distribution were expressed as ($\bar{x} \pm s$). Comparisons between groups were conducted using t-tests, and multiple groups were compared using ANOVA. Categorical data were expressed as counts (n) and percentages (%), with comparisons using χ^2-tests or Fisher's exact test. ROC curve analysis was used to evaluate diagnostic efficiency of individual and combined markers in early NSCLC screening; AUCs were compared with the DeLong test. Survival analysis was performed using Kaplan–Meier curves, and differences were assessed by the Log rank test. Cox proportional hazards model was used for multivariate prognostic analysis. A P-value <0.05 was considered statistically significant.

Results

IHC Expression of CD133 and OCT4

CD133 was primarily expressed on the cell membrane, while OCT4 was localized mainly in the nucleus. IHC results showed that the high expression rate of CD133 in NSCLC tissues was 66.3% (53/80), and for OCT4 it was 62.5% (50/80). In contrast, positive expression rates of CD133 and OCT4 in adjacent and control tissues were significantly lower, with statistical significance (P < 0.001), as shown in Table 3.

Table 3 Expression of CD133 and OCT4 in NSCLC, Adjacent, and Normal Tissues

Tissue Type	CD133 High Expression	OCT4 High Expression
NSCLC tissues (n=80)	53 (66.3%)	50 (62.5%)
Adjacent tissues (n=80)	14 (17.5%)*	11 (13.8%)*
Normal tissues (n=40)	6 (15.0%)*	4 (10.0%)*

Note: Compared with NSCLC tissues, *P < 0.05.

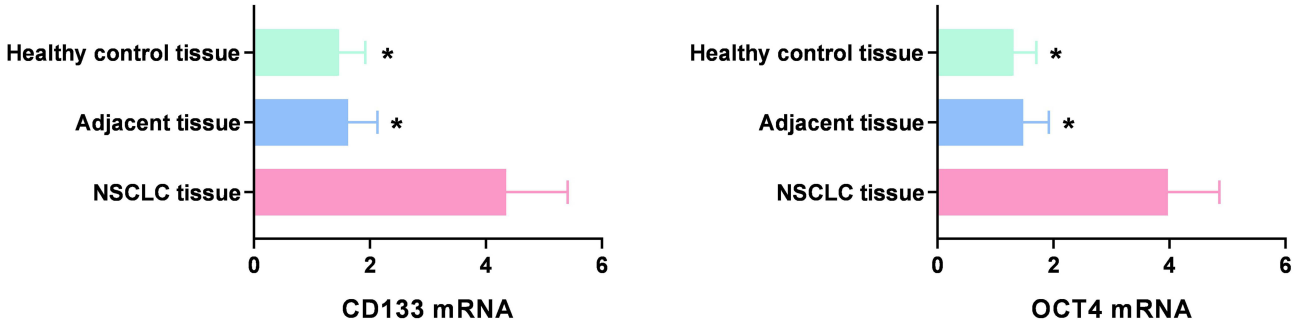

Figure 1 Comparison of CD133 and OCT4 Relative mRNA Expression Levels.
Note: Compared with NSCLC tissues, *P < 0.05.

mRNA Expression of CD133 and OCT4

qRT-PCR results showed significantly higher mRNA expression levels of CD133 and OCT4 in NSCLC tissues compared to adjacent tissues and healthy controls (F=312.25, 366.80; P < 0.001), as illustrated in Figure 1.

Relationship Between CD133 and OCT4 Expression and Clinicopathological Features

Based on IHC scoring, the 80 patients were grouped into CD133 high-expression (n=53) and low-expression (n=27), as well as OCT4 high-expression (n=50) and low-expression (n=30) groups. High expression of both CD133 and OCT4 was significantly associated with poor differentiation, larger tumor size, and presence of lymph node metastasis (P < 0.05). No significant differences were observed for other indicators (P > 0.05), as shown in Tables 4 and 5.

Table 4 Association Between CD133 Expression and Clinicopathological Features

Clinicopathological Feature	CD133		x^2	P
	High Expression (n=53)	Low Expression (n=27)		
Male	31 (58.5%)	15 (55.6%)	0.063	0.801
Age ≥60 years	29 (54.7%)	15 (55.6%)	0.005	0.943
Smoking history	36 (67.9%)	16 (59.3%)	0.590	0.442
Comorbidities	21 (39.6%)	10 (37.0%)	0.050	0.822
Adenocarcinoma type	33 (62.3%)	20 (74.1%)	1.115	0.290
Poor differentiation	41 (77.4%)	12 (44.4%)	8.666	0.003
Tumor diameter ≥3 cm	38 (71.7%)	12 (44.4%)	5.668	0.017
TNM stage IB–IIA	34 (64.2%)	9 (33.3%)	6.833	0.008
Lymph node metastasis	31 (58.5%)	8 (29.6%)	5.963	0.014
Vascular/pleural invasion	12 (22.6%)	4 (14.8%)	0.684	0.407
Post-op adjuvant therapy	35 (66.0%)	13 (48.1%)	2.385	0.122

Table 5 Association Between OCT4 Expression and Clinicopathological Features

Clinicopathological Feature	OCT4		x^2	P
	High Expression (n=50)	Low Expression (n=30)		
Male	30 (60.0%)	16 (53.3%)	0.341	0.559
Age ≥60 years	28 (56.0%)	13 (43.3%)	1.204	0.272
Smoking history	32 (64.0%)	20 (66.7%)	0.058	0.808
Comorbidities	20 (40.0%)	11 (36.7%)	0.087	0.767
Adenocarcinoma type	31 (62.0%)	22 (73.3%)	1.077	0.299

(*Continued*)

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Table 5 (Continued).

Clinicopathological Feature	OCT4		x^2	P
	High Expression (n=50)	Low Expression (n=30)		
Poor differentiation	39 (78.0%)	14 (46.7%)	8.232	0.004
Tumor diameter ≥3 cm	36 (72.0%)	14 (46.7%)	5.134	0.023
TNM stage IB–IIA	32 (64.0%)	11 (36.7%)	5.635	0.017
Lymph node metastasis	29 (58.0%)	10 (33.3%)	4.566	0.032
Vascular/pleural invasion	11 (22.0%)	5 (16.7%)	0.333	0.563
Post-op adjuvant therapy	34 (68.0%)	14 (46.7%)	3.555	0.059

Table 6 Diagnostic Efficacy of CD133 and OCT4 in Early NSCLC Screening

Marker	Cut-off Value	AUC	95% CI	P	Sensitivity (%)	Specificity (%)
CD133	4.15	0.809	0.730–0.887	<0.05	72.4	69.3
OCT4	3.80	0.796	0.713–0.879	<0.05	76.8	65.9
Combined	-	0.893	0.831–0.955	<0.05	88.7	82.5

Diagnostic Efficacy of CD133 and OCT4 in Early NSCLC Screening

The AUC for CD133 alone was 0.809, for OCT4 it was 0.796, and combined detection improved the AUC to 0.893, which was superior to either marker alone (Z_combined–CD133=3.354, P<0.05; Z_combined–OCT4=3.578, P<0.05), as shown in Table 6 and Figure 2.

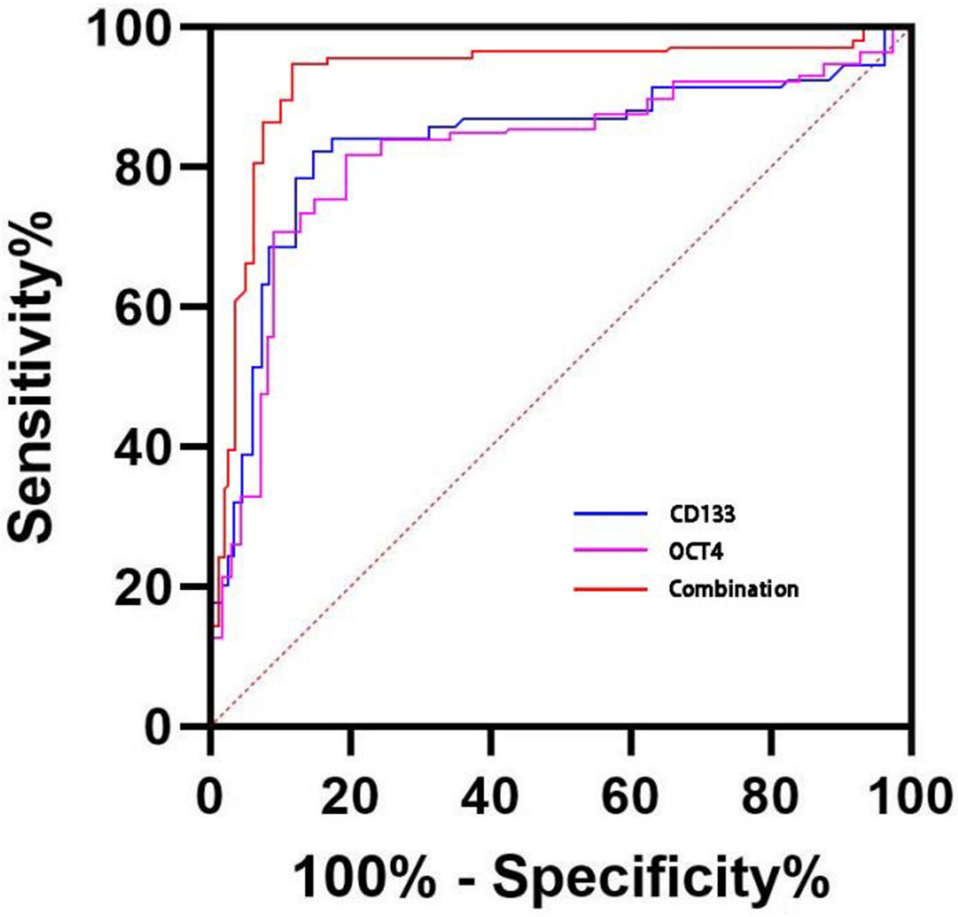

Figure 2 ROC Curves of CD133 and OCT4 for Early NSCLC Screening Diagnosis.

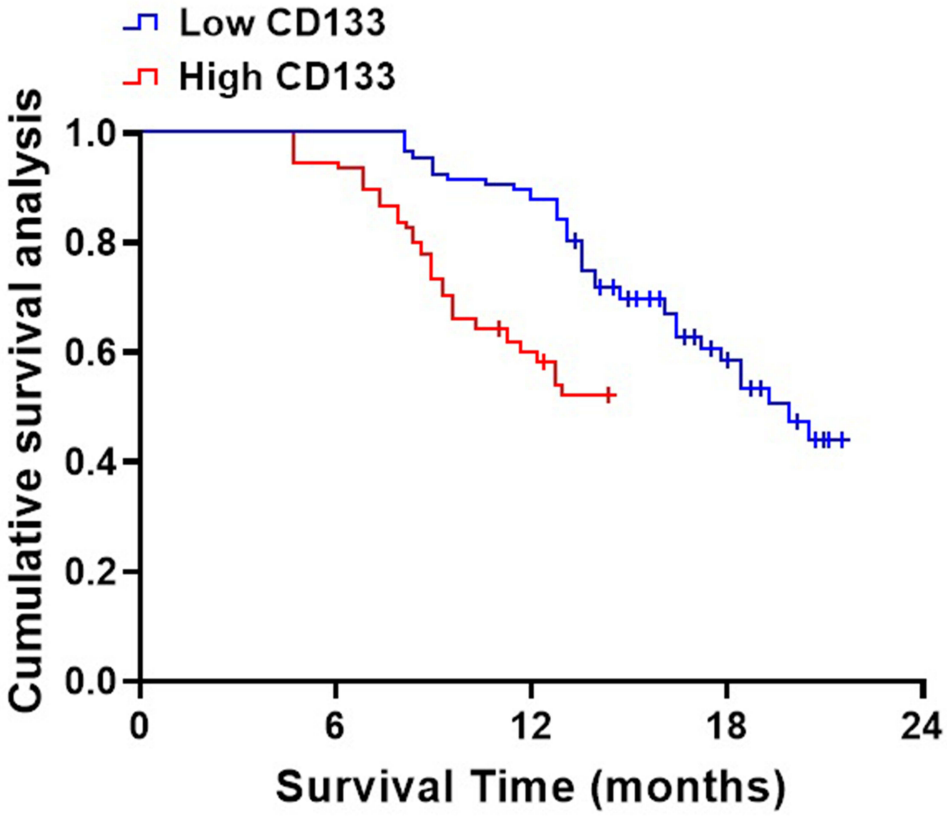

Figure 3 Kaplan–Meier Survival Curves for Different CD133 Expression Groups.

Survival Analysis

Postoperative follow-up was conducted for all 80 patients over a 24-month period, with a follow-up rate of 100.0%. A total of 21 death events occurred (26.3%). Kaplan–Meier survival analysis showed that the 2-year OS in the CD133 high expression group (n=53) was 66.0% (35/53), compared to 88.9% (24/27) in the CD133 low expression group, with significantly more deaths in the high expression group (Log rank test: χ^2=8.624, P=0.003), as shown in Figure 3. Similarly, the 2-year OS in the OCT4 high expression group (n=50) was 68.0% (34/50), compared to 90.0% (27/30) in the low expression group, with significantly lower overall survival in the high expression group (Log rank test: χ^2=7.352, P=0.007), as shown in Figure 4.

Analysis of Independent Risk Factors Affecting Prognosis

In this study, death within two years was set as the outcome variable, and the aforementioned clinical and pathological variables were used as independent variables to construct a Cox proportional hazards regression model. Based on preliminary univariate analysis (P<0.05 as entry criteria), the following six variables were included in the multivariate analysis: CD133 expression level, OCT4 expression level, tumor differentiation, tumor diameter, lymph node metastasis status, and TNM stage.Multivariate Cox regression analysis showed that high expression of CD133 and OCT4, poor tumor differentiation, larger tumor diameter, presence of lymph node metastasis, and TNM stage IB–IIA were independent risk factors affecting prognosis, as detailed in Table 7.

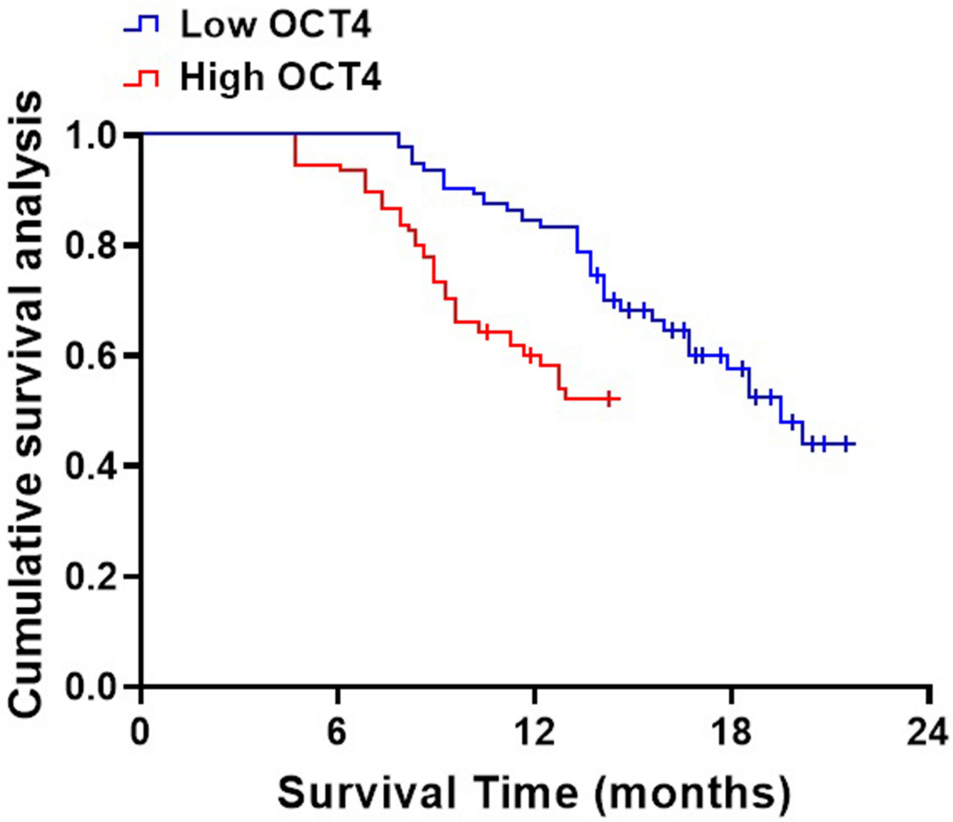

Figure 4 Kaplan–Meier Survival Curves for Different OCT4 Expression Groups.

Discussion

Based on analysis of tumor tissue samples from 80 early-stage NSCLC patients, this study found that the CSC markers CD133 and OCT4 were significantly overexpressed even in early lesions of NSCLC. Their expression levels were closely related to multiple adverse clinicopathological features. Combined detection of CD133 and OCT4 demonstrated high diagnostic efficacy in early NSCLC screening and could serve as independent prognostic markers. These findings provide potential molecular evidence for early diagnosis and individualized management of NSCLC, holding significant clinical translational value.

Early-stage NSCLC often lacks obvious symptoms and relies on imaging and histological methods for screening.[14] Recent studies indicate that CSCs, as the core subpopulation driving tumor heterogeneity and malignancy, are present even in early stages and participate in microenvironment modulation and immune evasion.[15,16]

CD133, a membrane glycoprotein, is widely considered a typical CSC marker, with its expression in lung cancer tissues being closely related to stemness and self-renewal capacity.[17,18] In this study, the positive expression rate of

Table 7 Independent Prognostic Risk Factors for Overall Survival in Multivariate Cox Regression Analysis

Variable	Category	HR	95% CI	P
CD133 Expression	High vs Low	2.45	1.38–4.36	0.003
OCT4 Expression	High vs Low	2.17	1.22–3.86	0.007
Tumor Differentiation	Poor vs Moderate/High	1.91	1.10–3.32	0.021
Tumor Diameter	≥3 cm vs <3 cm	1.84	1.03–3.27	0.039
Lymph Node Metastasis	Yes vs No	2.08	1.12–3.84	0.020
TNM Stage	IB–IIA vs IA	2.22	1.16–4.27	0.016

CD133 in NSCLC tumor tissues was significantly higher than in adjacent or healthy tissues, supporting its role in early tumorigenesis. OCT4 is a core transcription factor that maintains stem cell pluripotency and self-renewal and is also considered a critical "reprogramming" protein that induces cancer cell phenotype transformation.[19] Related studies[20,21] have shown that OCT4 can upregulate other stemness-related factors such as Nanog, SOX2, and KLF4, helping to maintain the CSC state. It also activates signaling pathways like PI3K/AKT and STAT3, thereby enhancing cell migration and drug resistance. In this study, OCT4 expression was positively correlated with CD133 to a certain extent, suggesting that the two may be co-expressed and jointly involved in CSC maintenance within lung cancer regulatory networks.

Previous research[22] suggests that single biomarkers often suffer from limitations in specificity or sensitivity, reducing their value in clinical screening. ROC analysis in this study showed that the combined detection of CD133 and OCT4 significantly improved early NSCLC detection, with AUC increasing from 0.809 and 0.796 to 0.893, indicating clear advantages of combined detection in molecular stratified diagnosis. In clinical practice, early-stage lung cancer patients often present with ground-glass nodules (GGNs) or subsolid lesions on imaging, making it difficult to determine malignancy.[23] If expression levels of CD133 and OCT4 can be noninvasively detected (eg, via liquid biopsy of peripheral blood circulating tumor cells or cell-free DNA), it could provide reliable support for imaging-based screening.

Survival analysis demonstrated that the 2-year OS rates in high expression groups of CD133 and OCT4 were significantly lower than those in the low expression groups, further confirming their characteristics as poor prognostic indicators. According to existing research, CSCs may influence prognosis in lung cancer through several mechanisms: (1) Enhanced anti-apoptotic capacity: CD133 and OCT4+ cells often upregulate anti-apoptotic proteins such as Bcl-2 and Survivin, rendering them resistant to chemo- and radiotherapy;[24,25] (2) Maintenance of quiescence: CSCs are often in the G0 phase, allowing them to escape cell cycle-dependent killing;[26,27] (3) Promotion of metastasis and microenvironment remodeling: CD133+ cells often express MMPs and VEGF, enhancing invasiveness and angiogenesis;[28] (4) Induction of immune evasion: OCT4 can upregulate PD-L1 expression and downregulate antigen-presenting molecules, making CSCs less recognizable to T cells.[29,30] Therefore, NSCLC patients with high expression of CSC markers are at higher risk of recurrence and death, even after radical surgery in early stages.

Previous clinical studies have confirmed the correlation between the expression of CD133 and OCT4 in lung cancer tissues and the malignancy of the tumor. For example, Wu, in a study on NSCLC patients, pointed out that high CD133 expression was associated with poor response to radiotherapy and shortened survival;[31] Koshimune, in early-stage lung adenocarcinoma patients, found that high OCT4 expression was related to poorer differentiation and higher risk of metastasis.[32] This study is the first to focus on stage I–IIA NSCLC, where the tumor has not yet undergone widespread invasion, and confirms that CSC markers already possess clear clinical diagnostic and predictive value at this stage, serving as a strong supplement to the aforementioned studies. The results of this study may serve clinical practice in the following ways: (1) Assist imaging-based early screening: In combination with LDCT screening, stratify high-risk populations through CD133/OCT4 detection in serum or biopsy tissue; (2) Postoperative risk prediction model development: Incorporate CD133/OCT4 into molecular pathology scoring systems to identify high-risk individuals for recurrence and optimize follow-up frequency; (3) Targeted therapy development: Develop small molecule inhibitors, antibody drugs, or siRNAs targeting OCT4 or CD133 to overcome CSC-related drug resistance; (4) Integrated immunotherapy evaluation: CSC expression may affect the efficacy of immune checkpoint inhibitors (such as PD-1/PD-L1), and may guide the timing and combination strategies of immunotherapy in the future.

NSCLC itself has high biological and molecular heterogeneity. Different pathological types (adenocarcinoma, squamous cell carcinoma, etc.) and genetic backgrounds may affect the expression patterns of CD133/OCT4. In addition, the limitations of this study should be acknowledged: (1) The sample size is relatively limited, which restricts the ability to conduct subtype analysis; (2) The single-center retrospective design may involve potential bias; (3) The lack of longitudinal interventional observation prevents confirmation of the dynamic relationship between expression changes and disease progression; (4) The lack of protein functional experiments makes it impossible to further verify regulatory pathways and mechanisms of action. Therefore, while our findings are consistent with the study objectives and provide meaningful insights into the early diagnosis and prognosis of NSCLC, future research should be expanded through larger multi-center, prospective studies combined with functional validation to further confirm their broader clinical applicability.

Conclusion

The results of this study indicate that the cancer stem cell markers CD133 and OCT4 are significantly overexpressed in tumor tissues of early-stage NSCLC patients, and their expression levels are closely associated with poor tumor differentiation, larger tumor diameter, presence of lymph node metastasis, and advanced TNM stage. The combined detection of the two markers demonstrates higher diagnostic efficacy than either marker alone, significantly improving the identification capability for early-stage NSCLC. Furthermore, survival analysis and Cox regression models confirm that high expression of both CD133 and OCT4 are adverse prognostic factors affecting overall survival, with good prognostic predictive value. This study suggests that CD133 and OCT4 not only have potential value as molecular markers for early NSCLC screening but may also serve as important bases for auxiliary risk stratification and the development of individualized treatment strategies. Nevertheless, the relatively small sample size and single-center retrospective design should be acknowledged as limitations, and future studies integrating larger multicenter cohorts, dynamic monitoring, functional exploration, and clinical translation could further validate and promote their application in the precision diagnosis and treatment of lung cancer.

Disclosure

The authors report no conflicts of interest in this work.

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