Prognostic significance of NDRG2 combined with EGFR patients with lung adenocarcinoma

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Abstract

Background: N-myc downstream-regulated gene 2 (NDRG2) plays a substantial role in lung adenocarcinoma (LUAD). Epidermal growth factor receptor (EGFR) mutation could significantly improve prognosis in patients with LUAD. In this study, we aimed to elucidate the prognostic value of NDRG2/EGFR in patients with LUAD.

Methods: Immunohistochemistry, western blotting, and real-time polymerase chain reaction (RT-PCR) were conducted to detect the expression levels of NDRG2 protein. Associations between NDRG2/EGFR expression and clinicopathological characteristics of patients with LUAD were examined as well. Serum levels of carcinoembryonic antigen (CEA) were tested prior to treatments. Patients' overall survival (OS) was assessed by the Kaplan-Meier method. Multivariate Cox regression analysis was carried out to investigate the effects of patients' demographic characteristics on overall survival .

Results: The expression of NDRG2 was significantly decreased in patients with LUAD. The expression of NDRG2 was positively correlated with the levels of CEA and EGFR. Advanced stages were significantly associated with low expression of NDRG2. We found that the patients in the NDRG2-high/EGFR(+) group had the best outcomes, while the patients in the NDRG2-low/EGFR(-) group had the worst outcomes. Cox regression analysis showed that NDRG2-low/EGFR(+), NDRG2-high/EGFR(+), and vascular invasion were independent prognostic factors of LUAD.

Conclusion: NDRG2 and EGFR should be considered in patients with LUAD.

Background

Lung cancer remains the leading cause of cancer-related death worldwide, accounting for 19.4% of cancer mortality among adults [1]. Among lung cancers, small-cell lung cancers represent approximately 15% of the cases and non-small cell lung cancers (NSCLCs) approximately 85%. NSCLC includes lung adenocarcinoma (LUAD), which is the most prevalent subtype of lung cancer [2]. Only approximately 20% of patients with NSCLC can be potentially treated by resection, and the remaining are diagnosed in advanced stages [3]. Despite advances accomplished in terms of early detection and standard treatments such as surgery, chemotherapy, radiotherapy, and iodine-125 (^{125}I) brachytherapy, the overall survival (OS) of NSCLC still remains poor [3, 4].

Targeted therapy, such as tyrosine kinase inhibitors (TKIs), has recently emerged as a new therapeutic approach for patients with advanced NSCLC, especially for LUAD harboring EGFR mutations, showing better progression-free survival (PFS) compared with wild type tumors [5, 6]. Nevertheless, after surgical resection, several patients eventually relapse due to inevitable drug resistance, and patients lacking driver oncogene aberrations are still treated with traditional regimens, with poor outcomes [7]. Hidayat et al. found that FBXW7 expression in CD133-positive cells was increased, and c-MYC expression was decreased in gefitinib-resistant tumors from PC9 cells in mice and in nine out of 14 tumor specimens from patients with EGFR-mutant NSCLC and acquired resistance to gefitinib [8]. Another study reported that hyperprogressive disease (HPD) promptly leads to death in patients harboring EGFR exon 20 insertion mutation and MYC amplification [9]. Consequently, the interaction between MYC and EGFR may play a significant role in malignant tumor cells, involving cell proliferation, invasion, and metastasis.

The N-myc downstream-regulated gene (NDRG) family consists of four members: NDRG1, NDRG2, NDRG3, and NDRG4 [10]. A previous study showed that NDRG2 exerts important functions in cell differentiation and tumor suppression. Researches revealed that DNA damage, hypoxia, and glucocorticoids promoted NDRG2 expression,

and NDRG2 can be transcriptionally activated by p53 and HIF1-α [11]. Decreased expression of NDRG2 has been found in several types of human cancers, such as lung cancer, bladder cancer, colon cancer, pancreatic cancer, thyroid cancer, glioblastoma, melanoma, and meningioma; besides, NDRG2 was found as a candidate tumor suppressor gene [12]. Although a previous analysis revealed that NDRG2 might serve as a novel prognostic marker in human lung cancer, its prognostic value in LUAD needs to be further elucidated. In addition, the association between NDRG2 expression and EGFR mutation still remains elusive.

Accordingly, the identification of novel prognostic markers for LUAD is urgently required. The present study examined the prognostic value of the combined detection of NDRG2/EGFR in patients with LUAD.

Methods

Patients

A total of 89 LUAD patients were prospectively enrolled in this cohort study at the Tianjin First Central Hospital of Nankai University between June 2013 and June 2014 (52 men and 37 women; mean age, 65.6 years old; range of age, 38-86 years old). None of the patients had received chemotherapy, radiotherapy, and/or immunotherapy before sampling. The clinical and pathologic characteristics of the patients with LUAD are listed in Table 1. Those individuals who smoked at least 1 cigarette per day for over 1 year were defined as smokers. Tumor staging and grading were according to the 8th edition of the Union for International Cancer Control (UICC) TNM classification of malignant tumors. Hematoxylin and eosin (H&E) staining of tissue slides was performed and verified by two board-certified pathologists. Among the patients, 34 cases underwent curative-intent surgery; in addition, 55 patients with advanced LUAD were treated with iodine-125 (^{125}I) brachytherapy. Patients' preoperative workup (e.g., positron emission tomography (PET)/computed tomography (CT), cardiac ultrasound, and lung function) was examined to exclude those cases with secondary lung cancer and those with systemic disease (Figure 1). Normal tissues were removed from at least 5 cm away from the edge of the tumors.

Immunohistochemistry

In the present study, formalin-fixed paraffin-embedded tissue sections (thickness, 4-μm) were used for detecting the expression of NDRG2. Tissue sections were dewaxed, rehydrated, antigen-retrieved, and cooled to room temperature. The sections were incubated with mouse monoclonal anti-NDRG2 antibody (Abcam, Cambridge, UK) at 4ºC overnight, rinsed with phosphate-buffered saline (PBS), and incubated with horseradish peroxidase (HRP)-labeled goat anti-mouse secondary antibody for 60 min. NDRG2 expression was revealed using 3,3'-diaminobenzidine (DAB) as the chromogen. Negative control was performed by replacing the primary antibody with normal mouse serum. The brown or yellow staining was identified as a positive expression. The total staining score of 0-12 was considered in a semi-quantitative manner and stratified as follows: negative (-, range of score: 0-1), weak (+, range of score: 2-4), moderate (++, range of score: 5-8), or strong (+++, range of score: 9-12). The tumor specimens were divided into the low-expression group (range of score: 0-4) and the high-expression group (range of score: 5-12) [12].

Western blot analysis

Total protein concentration was measured by the bicinchoninic acid (BCA) assay kit. The proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were incubated with mouse anti-human NDRG2

antibody (1:500, Abcam, Cambridge, MA, USA), CyclinD3 (1:1000, Cell Signaling Technology, Inc., Danvers, MA, USA), SOCS1 (1:500, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and GAPDH (1:1000, Beyotime Institute of Biotechnology, Haimen, China) at 4°C overnight after being blocked with 5% non-fat milk for 1 h. β-actin was used as internal control. The membranes were washed and incubated with HRP-conjugated secondary antibody (Santa Cruz Biotechnology Inc., Dallas, TX, USA). The blots were visualized using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech, Arlington Heights, IL, USA) according to the manufacturer's instructions. Each experiment was performed in triplicate.

Real-time polymerase chain reaction (RT-PCR)

The total RNA was extracted from the fresh tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The sequences of the primers were: NDRG2, forward-5'-ATG GCG GAG CTG CAG GAG GTC-3', and reverse-5'-AAC AAG GGC CAT TCA ACA GGA GAC-3'; GAPDH, forward-5'-GCC TCA AGA TCA GCA AT-3' and reverse-5'-AGG TCC ACC ACT GAC ACG TT-3'. The RT-PCR conditions were: denaturation at 94ºC for 30 s, annealing at 58ºC for 30 s, and extension at 72ºC for 40 s. The relative mRNA expression of NDRG2 was calculated by the $-2^{\Delta\Delta Ct}$ method. Each experiment was undertaken in triplicate.

Measurement of parameters

Blood samples were collected before initiation of treatment. Patients' blood type was examined by measuring the serum concentrations. CEA was measured routinely at the hospital's central laboratory with human carcinoembryonic antigen ELISA kit (Abcam) .

Statistical analysis

Categorical data were expressed as frequencies or percentages. Continuous data were presented as means ± standard deviations (SD). Differences between categorical groups were investigated by the chi-square test or Fisher's exact test. The differences in means between groups were analyzed using the Mann-Whitney U-test or Student's t-test. Associations between two variables were quantified using Spearman's rank correlation coefficient. The OS rate was evaluated using the Kaplan-Meier method, and differences between the groups were assessed using the log-rank test. Multivariable analysis was conducted using Cox's proportional hazards regression model to investigate the effects of patients' demographic characteristics on OS. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant.

Results

NDRG2 was downregulated in LUAD, SOCS1, and CyclinD3 compared with normal tissues

NDRG2 protein was mainly found in the cytoplasm, and a weak expression could be found in a limited number of cell nuclei (Figure 2 A-C). The expression of NDRG2 at the protein (Figure 2 D) and mRNA (Figure 2 E) levels in LUAD was significantly lower compared with normal tissues.

To evaluate the expression of NDRG2, SOCS1, and CyclinD3 in different stages of LUAD, we divided 89 pairs of normal and LUAD samples into four groups according to the disease stage. There were 24, 15, 10, and 40 samples in stages I, II, III, and IV, respectively. The expression of NDRG2 and SOCS1 gradually decreased as the

LUAD stage increases (Figure 3 B-C), while the expression of CyclinD3 gradually increased as the LUAD stage increases (Figure 3 D).

Relationship between the expression of NDRG2 and clinicopathological features of patients with LUAD

As shown in Figure 4, the expression level of NDRG2 was associated with CEA ($P < 0.001$).

As shown in Table 2, the expression level of NDRG2 was notably higher in LUAD tissues in stages I-II than that in stage III-IV ($P<0.001$). In addition, the frequencies of no vascular invasion and EGFR positivity (+) were significantly higher in patients with high expression of NDRG2 than that in patients with low expression of NDRG2 ($P<0.001$ and 0.001, respectively). There were no associations between expression levels of NDRG2 and other clinicopathological features, including age, sex, smoking history, and blood type ($P>0.05$).

Regarding the 34 patients who underwent surgery, the expression level of NDRG2 was significantly higher in stage I-II than that in stage III-IV ($P=0.028$). The frequencies of no vascular invasion and EGFR positivity (+) were higher in patients with high expression of NDRG2 than that in the patients with low expression of NDRG2 (0.008 and 0.030, respectively).

Prognostic implications of NDRG2 and EGFR expression

Based on the clinicopathological features of the patients with LUAD, as well as the expression levels of NDRG2, EGFR, and CEA, the survival was analyzed by the Kaplan-Meier method (Figure 5). The results showed that iodine-125 radioactive seeds brachytherapy for advanced LUAD with high expression level of NDRG2 led to significantly higher OS than in LUAD with low expression level ($P = 0.0261$, Figure 5 A). patients with LUAD, EGFR(+), and CEA < 2.0 ng/ml had higher OS ($P < 0.0001$, 0.0314, Figure 5 B-C). In addition, in operated patients with high expression of NDRG2 (Figure 5 E), EGFR(+) (Figure 5 F), and CEA < 2.0 ng/ml (Figure 5 G), higher OS was noted ($P = 0.0022$, < 0.0001 and 0.013, respectively).

According to the conjoined expressions of NDRG2/EGFR, the subjects were categorized into four groups: NDRG2-low/EGFR-negative(-), NDRG2-low/EGFR-positive(+), NDRG2-high/EGFR-negative(-), and NDRG2-high/EGFR-positive(+). The association between the co-expression of NDRG2/EGFR and OS was tested by the Kaplan-Meier method. In these four groups, iodine-125 radioactive seeds brachytherapy for advanced LUAD patients in the NDRG2-high/EGFR(+) group was accompanied by the best prognosis during the 5-year follow-up ($P < 0.0001$, Figure 5 D), and the same results were observed in operated patients ($P = 0.0002$, Figure 5 H).

Cox regression analysis

As shown in Table 3, NDRG2-low/EGFR(+) (hazard ratio (HR)=6.508; 95% confidence interval (CI), 2.619-16.174; $P<0.001$), NDRG2-high/EGFR(+) (HR=3.519; 95% CI, 1.384-8.949; $P=0.008$), and vascular invasion (HR=4.480; 95%CI, 2.291-8.760; $P<0.001$) were independent prognostic factors of OS.

Discussion

To improve the prediction of lung cancer survival, several tumor markers (e.g., CEA) have been assessed and extensively used [13, 14], but each marker has its own specificity and sensitivity, which might lead to limitations in prognostic ability. The combined detection of tumor markers may be of great importance for improving the prediction of lung cancer survival.

MYC influences growth, proliferation, differentiation, and apoptosis of cancer cells through regulating the expression of numerous genes, including SOCS1 and CyclinD3 [15]. In addition, MYC governs events associated with tumor progression, including genetic stability, migration, and angiogenesis [16]. Two human cDNAs, encoding NDRG3 and NDRG4, are homologous to NDRG1. These two genes, together with NDRG1 and a previously deposited cDNA (designated NDRG2), constitute the NDRG gene family [17]. Previous studies reported that NDRG2 was associated with human lung cancer, and the decreased expression of NDRG2 was correlated with a worse outcome of lung cancer patients [12, 18]. Similarly, the results of the present study showed that the high expression level of NDRG2 in LUAD patients was significantly associated with the early TNM stage and negative vascular invasion. The low expression level of NDRG2 showed a lower OS than the high expression level of NDRG2. The above-mentioned findings indicate that NDRG2 may play a pivotal role in the development of LUAD. Accordingly, the expression of SOCS1, a negative regulator of cytokine signaling, was decreased along with increasing tumor stage, while the expression of CyclinD3, which is involved in cell cycle progression, was increased with increasing tumor stage. Those results are supported by previous studies in lung cancer [19, 20].

CEA was first described in 1965 by Gold and Freedman as an antigen present in gastrointestinal carcinoma cells [14, 21]. A number of researches demonstrated the prognostic value of preoperative CEA levels as a classical marker for LUAD [22, 23]. In the present study, we, for the first time, showed that CEA levels > 2.0 ng/ml were associated with the low expression level of NDRG2, and a lower OS was found compared with CEA levels < 2.0 ng/ml. The study indicates that CEA levels < 2.0 ng/ml might indicate a good prognosis for LUAD.

Research revealed that EGFR mutations in circulating tumor DNA (ctDNA) predicted a better PFS, in particular in advanced patients with NSCLC treated by EGFR-TKIs. KRAS mutations in ctDNA indicated a worse PFS and OS in patients treated by chemotherapy [24]. Another study demonstrated that blood, in particular serum, is an appropriate substitute when tumor tissue is absent or insufficient for testing EGFR mutations to guide EGFR-TKIs treatment in patients with NSCLC [25]. In the present research, we reported the EGFR mutation status in patients with adenocarcinoma and its correlation with the expression level of NDRG2. Mutant EGFR expression was positively correlated with higher OS in the presence of a high expression level of NDRG2.

MYC and EGFR have been identified as potential biomarkers that can predict the efficacy of targeted therapy [26, 27]. We, herein, analyzed the prognostic value of the combined detection of NDRG2 and EGFR for LUAD. We found that the patients in the NDRG2-high/EGFR(+) group had the best outcome, while the patients in the NDRG2-low/EGFR(-) group had the worst. Cox regression analysis revealed that NDRG2-low/EGFR(+), NDRG2-high/EGFR(+), and vascular invasion were independent prognostic factors of OS (Table 3).

This study has several limitations. First, it was a retrospective study performed at a single center. Second, the sample size was very limited. The present study, in itself, cannot be used to change clinical practice and to propose the use of a new test. Those results have to be validated using a large sample size. Hence, further study is required to explore the putative association between NDRG2/EGFR level and OS in patients with NSCLS.

Conclusions

In summary, this study reported the different expression levels of NDRG2 in patients with LUAD. In addition, for the first time, the relationship between NDRG2/EGFR expression and clinicopathological characteristics of patients with LUAD, especially prognosis status, was investigated. NDRG2/EGFR can be used as a novel prognostic biomarker for patients with LUAD.

Abbreviations

NDRG2: N-Myc downstream-regulated gene2

LUAD: Lung adenocarcinoma

EGFR: Epidermal growth factor receptor

CEA: Carcinoembryonic antigen

NSCLC: Non-small cell lung cancer

TKIs: Tyrosine kinase inhibitors

HPD: Hyperprogressive disease

HIF1-α: Hypoxia-inducible factor (HIF)1-α

PBS: Phosphate-buffered saline

DAB: 3,3'-Diaminobenzidine

SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

PVDF: Polyvinylidene fluoride

RT-PCR: Reverse transcription-polymerase chain reaction

HRP: Horseradish peroxidase

SOCS1: Suppressor of cytokine signaling 1

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

SD: Standard deviation

ctDNA: circulating tumor DNA

TNM: Tumor lymph node metastasis

OS: Overall survival

PFS: Progression-free survival

SE: Standard error

HR: Hazard ratio

CI: Confidence intervals

Declarations

Acknowledgment

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Authors' contributions

BY, XPL, TJ, and WDZ conceived of the study. HGZ, TX, and XHL performed data analysis for experiments. BY, HGZ, TX, and LL drafted the final version of the manuscript and figure legends. BY, XPL, XHL, and LZ revised the figures, added critical content to the discussion, and were responsible for revising all portions of the submitted portion of the manuscript. TX and XHL performed the experiments using lung adenocarcinoma and control tissue. All contributors meet the criteria for authorship. All of the authors read and approved the final manuscript.

Ethical approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and was confirmed by the Ethics Committee of Tianjin First Central Hospital of Nankai University (Tianjin, China; approval no. 2018N054KY). All patients signed the written informed consent forms.

Consent for publication

Not applicable

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Availability of data and materials

The data of the current research are available from the corresponding author on a reasonable request.

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Tables

Table 1. Patients' demographic characteristics.

Clinical characteristics	Total n	%
Age, years		
≤65	42	47.2
>65	47	52.8
Sex		
Male	52	58.4
Female	37	41.6
Smoking status		
Non-smoker	41	46.1
Smoker	48	53.9
Blood type		
A	29	32.6
B	27	30.3
O	24	27.0
AB	9	10.1
Lobe location		
Right	52	58.4
Upper lobe of right lung	30	57.7
Middle lobe of right lung	5	9.6
Inferior lobe of right lung	12	23.1
Center-type of right lung	5	9.6
Left	37	41.6
Upper lobe of left lung	21	56.8
Inferior lobe of left lung	13	35.1
Center-type of left lung	3	8.1
T		
1a	9	10.1
1b	11	12.4
1c	18	20.2
2a	21	23.6
2b	13	14.6
3	7	7.9
4	10	11.2
N		
0	29	32.6
1	20	22.5
2	27	30.3
3	13	14.6
M		
0	49	55.1
1a	20	22.5
1b	6	6.7
1c	14	15.7
Stage		
I	24	27.0
II	15	16.9
III	10	11.2
IV	40	44.9
Vascular invasion		
No	38	42.7
Yes	51	57.3
EGFR		
positive	31	34.8

negative	58	65.2

T, tumor; N, node; M, metastasis; EGFR, epidermal growth factor receptor

Table 2. Patients' clinicopathological characteristics according to NDRG2 level.

Parameters	Total patients (n=89)			Operated patients (n=34)		
	NDRG2 low group	NDRG2 high group	P-value	NDRG2 low group	NDRG2 high group	P-value
Gender			0.988			0.710
Male	31	21		6	15	
Female	22	15		5	8	
Age (years)			0.996			0.705
<65	25	17		3	9	
≥65	28	19		8	14	
Smoking status			0.771			0.717
Non-smoker	19	14		5	13	
Smoker	34	22		6	10	
Blood type			0.265			0.905
A	15	14		5	9	
B	20	7		1	4	
AB	4	5		2	5	
O	14	10		3	5	
Stage			<0.001			0.072
I	6	18		5	16	
II	6	9		3	7	
III	8	2		2	0	
IV	33	7		1	0	
			<0.001			0.028
I+II	12	27		8	23	
III+IV	41	9		3	0	
CEA			<0.001			<0.001
<2.0	4	24		0	14	
≥2.0	49	12		11	9	
Vascular invasion			<0.001			0.008
No	19	32		6	22	
Yes	34	4		5	1	
EGFR			0.001			0.030
Negative(-)	42	16		9	9	
Positive(+)	11	20		2	14	

NDRG2, N-Myc downstream-regulated gene2; CEA, carcinoembryonic antigen (ng/ml); EGFR, epidermal growth factor receptor.

Table 3. Prognostic value of conjoined expression of NDRG2/EGFR by multivariate Cox regression analysis.

	B	SE	P-value	HR	95.0 % CI for Exp(B)	
					Lower	Upper
Age	0.080	0.261	0.760	0.923	0.554	1.539
Sex	-0.171	0.317	0.590	1.186	0.637	2.209
Smoking status	0.066	0.309	0.831	0.936	0.511	1.716
Lobe location	0.186	0.262	0.479	0.831	0.497	1.388
Vascular invasion	1.500	0.342	<0.001	4.480	2.291	8.760
NDRG2-low/EGFR(-)			<0.001			
NDRG2-low/EGFR(+)	1.873	0.464	<0.001	6.508	2.619	16.174
NDRG2-high/EGFR(-)	0.576	0.556	0.300	1.779	0.598	5.292
NDRG2-high/EGFR(+)	1.258	0.476	0.008	3.519	1.384	8.949

Figures

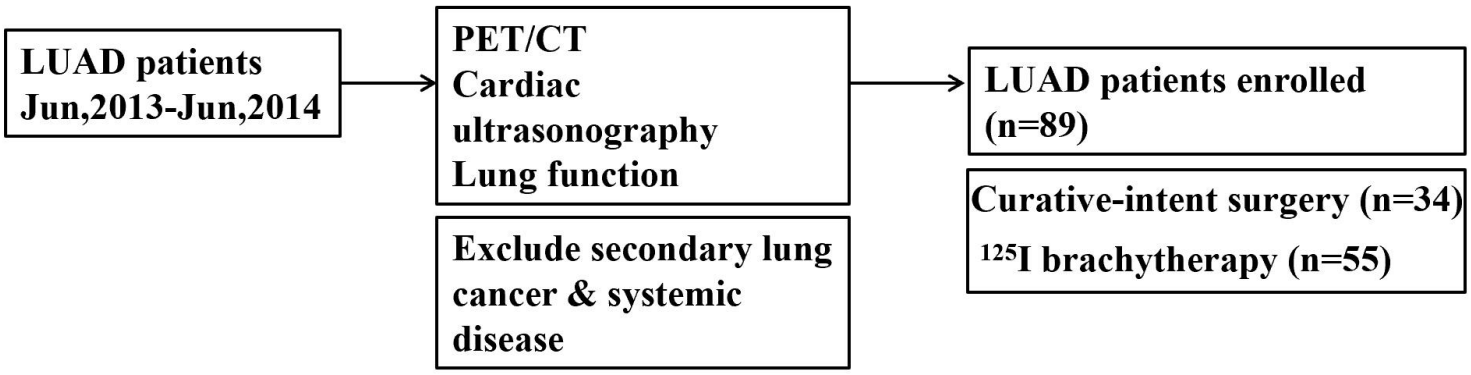

Figure 1

Patient flowchart. Abbreviations: LUAD lung adenocarcinoma; PET positron-emission tomography; CT computed tomography; 125I iodine-125

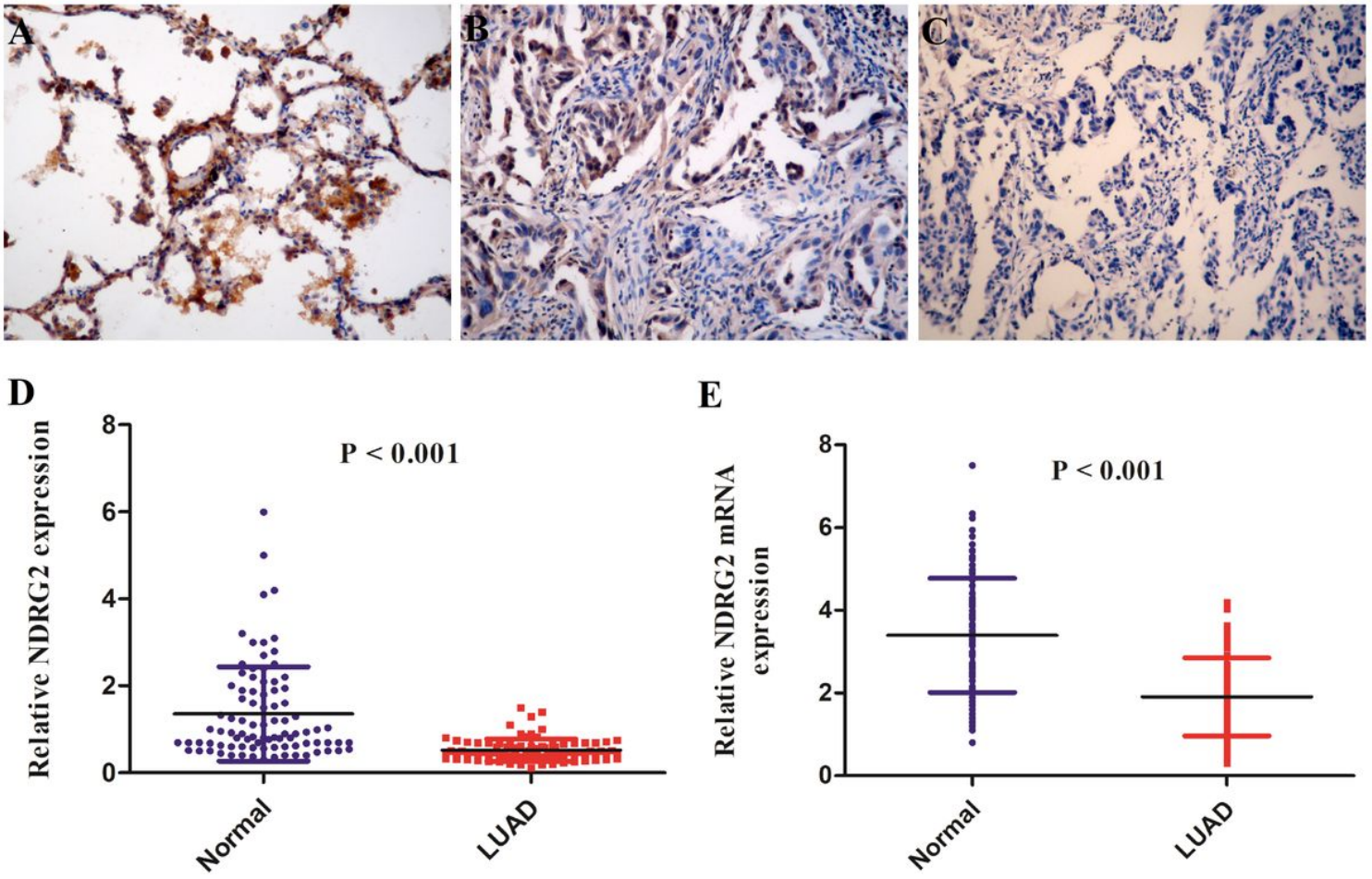

Figure 2

The expression level of NDRG2 in LUAD patients and normal tissues (Figure 2A-E). Immunohistochemistry showed the expression level of NDRG2 in normal lung tissues (Figure 2A), LUAD patients (Figure 2B), and negative control (Figure 2C) (200× magnification). The expression level of NDRG2 protein was determined by western blot assay (Figure 2D) and RT-PCR (Figure 2E). It was significantly downregulated in patients with LUAD compared with that in normal tissues at both protein and mRNA levels (*P < 0.001). Abbreviations: NDRG2, N-Myc downstream-regulated gene 2; LUAD, lung adenocarcinoma; RT-PCR, reverse transcription-polymerase chain reaction.

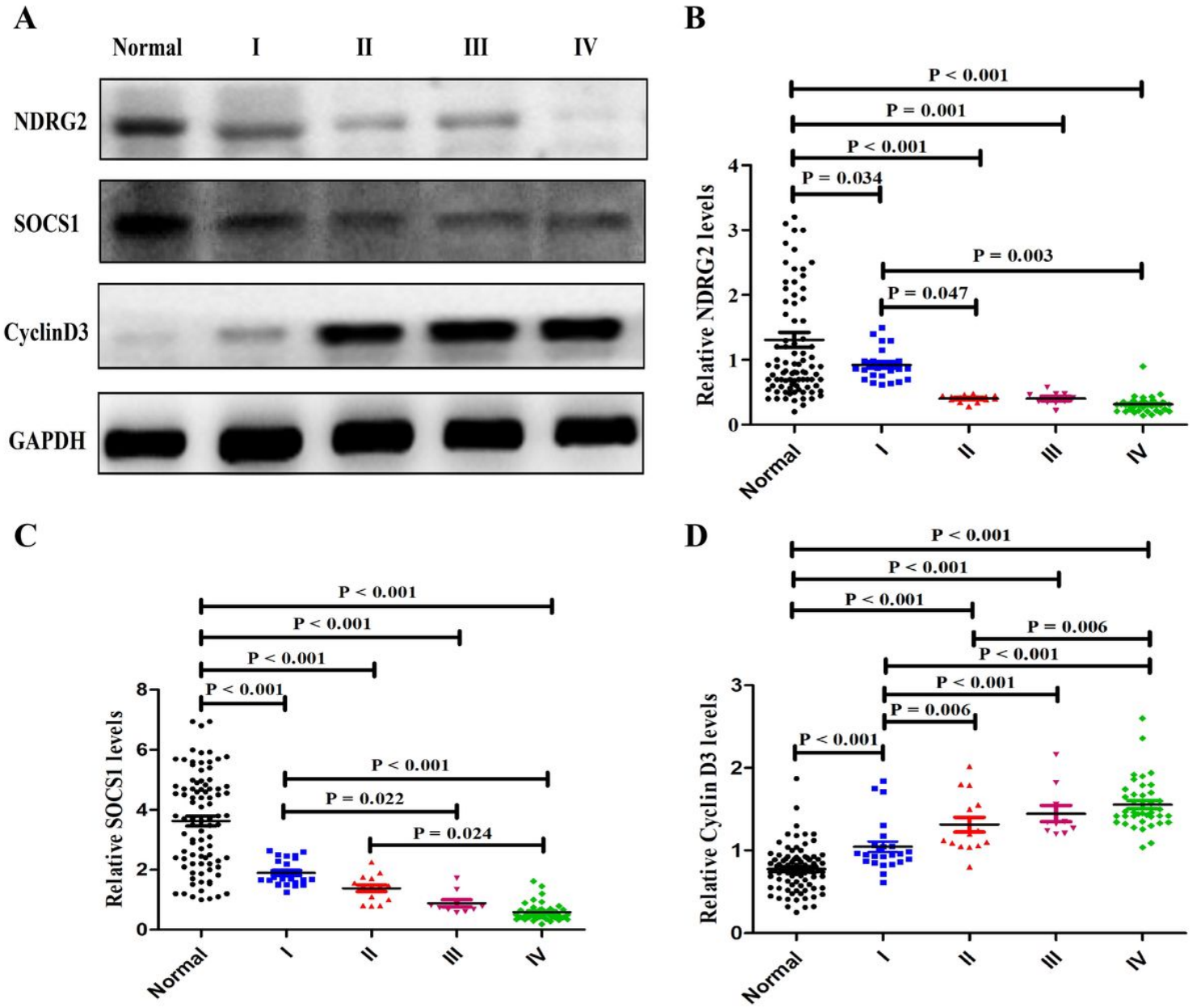

Figure 3

Associations between the expression levels of NDRG2 and clinicopathological features of patients with LUAD. The NDRG2, SOCS1, and CyclinD3 levels were evaluated by western blot (Figure 3A-D). The NDRG2 and SOCS1 levels decreased with the increasing LUAD stage, while the cyclinD3 levels increased with the increasing LUAD stage. Abbreviations: NDRG2, N-Myc downstream-regulated gene 2; LUAD, lung adenocarcinoma; SOCS1, suppressor of cytokine signaling 1; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

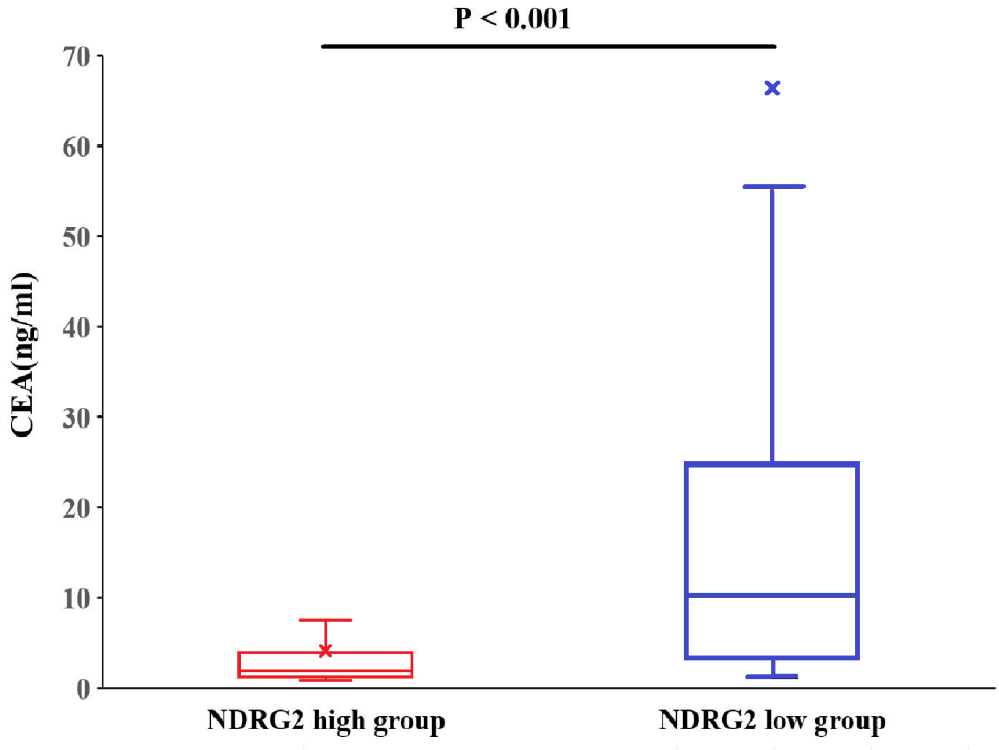

Figure 4

Associations between the expression levels of NDRG2 and CEA levels of patients with LUAD. The CEA levels were notably lower in patients with a high expression level of NDRG2 than that in patients with the low expression level of NDRG2 (P < 0.001). Abbreviations: CEA, carcinoembryonic antigen; NDRG2, N-Myc downstream-regulated gene 2; LUAD, lung adenocarcinoma.

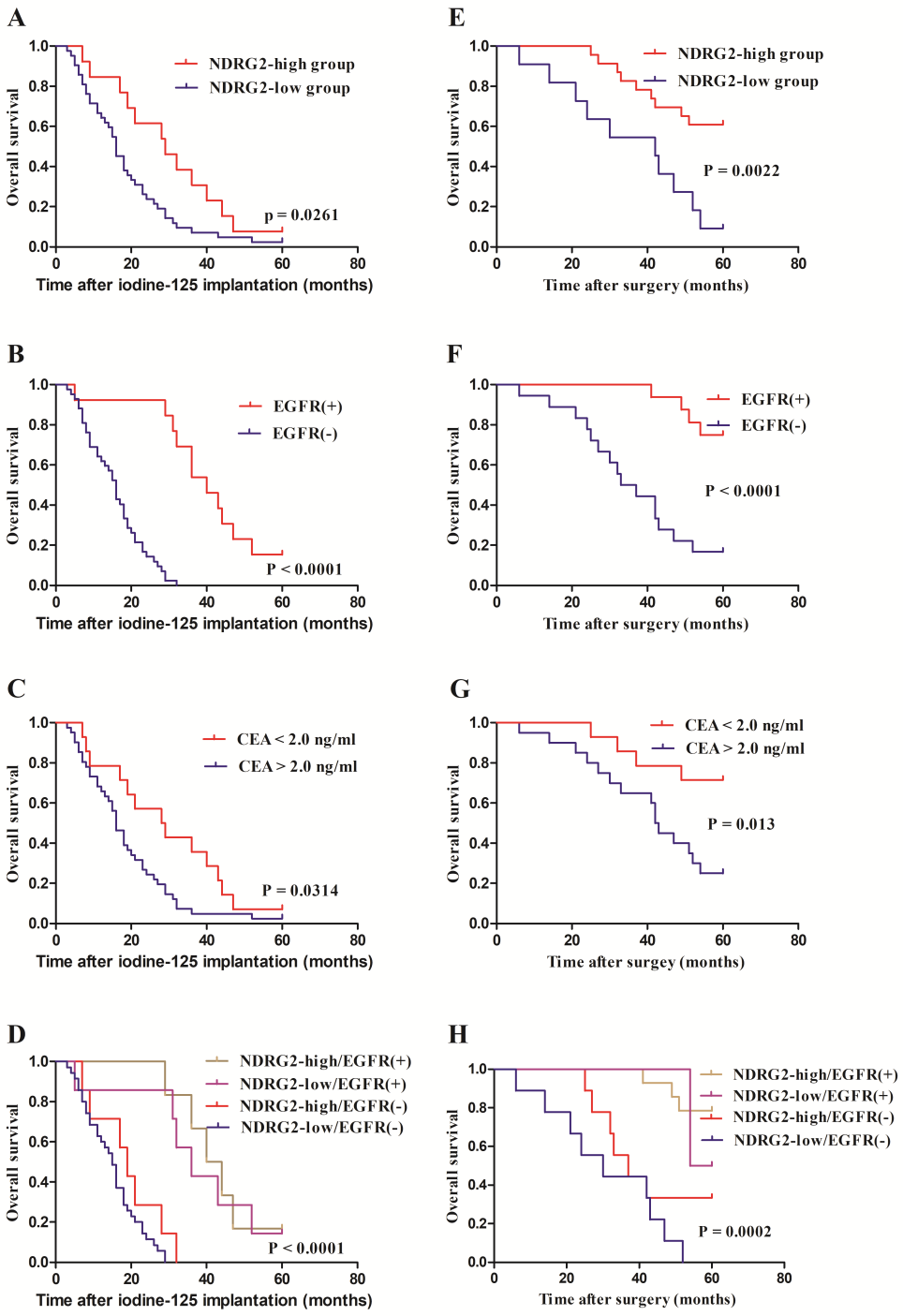

Figure 5

Overall survival of patients with LUAD (Figure 3A-H). Overall survival of patients with advanced LUAD treated with iodine-125 radioactive seeds brachytherapy (Figure 3A-D). The patients with low expression level of NDRG2 (P = 0.0261, Figure 3A), negative EGFR expression (P < 0.0001, Figure 3B), and CEA > 2.0 ng/ml (P = 0.0314, Figure 3C) exhibited significantly lower overall survival rates. According to the conjoined expressions of NDRG2/EGFR, the subjects were categorized into four groups: NDRG2-low/EGFR-negative(-), NDRG2-low/EGFR-positive(+), NDRG2-high/EGFR-negative(-), and NDRG2-high/EGFR-positive(+). Patients with co-expression of NDRG2-low/EGFR-negative(-) had the worst outcome of overall survival among the four groups (P < 0.0001, Figure 3D). Overall survival of operated patients (Figure 3E-H). The patients with low expression level of NDRG2 (P = 0.0022, Figure

3E), negative EGFR expression (P < 0.0001, Figure 3F), and CEA > 2.0 ng/ml (P = 0.013, Figure 3G) exhibited remarkably lower overall survival rates. Patients with co-expression of NDRG2-low/EGFR-negative had the worst outcome for overall survival among the four groups (P = 0.0002, Figure 3H). Abbreviations: LUAD, lung adenocarcinoma; NDRG2, N-Myc downstream-regulated gene2; CEA, carcinoembryonic antigen; EGFR, epidermal growth factor receptor.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- originalSOCS1.jpg
- originalCyclinD3GAPDH.jpg
- originalNDRG2.jpg