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Original Research Article

Integrative analysis of inhibitor of DNA-binding expression and prognosis in non-small cell lung cancer

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Abstract

Purpose: *To investigate the expression and prognosis of inhibitor of DNA-binding (ID) protein family in non-small cell lung cancer (NSCLC).*
Methods: *The mRNA expression and prognostic relevance of ID family using Oncomine, UALCAN, Kaplan-Meier plot, and The Cancer Genome Atlas (TCGA) databases were compiled and analyzed. Also, associations between individual IDs' mRNA expression and clinicopathological features in NSCLC patients were identified using unpaired t-test.*
Results: *There was significantly higher expression of each ID in normal cells compared to NSCLC tissues, including adenocarcinoma (AC) and squamous cell carcinoma (SCC; $p < 0.05$). High ID1 mRNA expression correlated significantly with worse overall survival in NSCLC, AC, and SCC cases ($p < 0.05$). Also, increased ID4 mRNA expression predicted significantly better overall survival in NSCLC and AC cases, but not in SCC ($p < 0.05$).*
Conclusion: *Inhibitor of DNA-binding 1 mRNA predicts poorer survival, whereas ID4 mRNA suggests better survival, particularly in AC. ID2 and ID3 mRNA levels lack significant associations with overall survival, suggesting targeting IDs might represent potential therapeutics for NSCLC. There is the need for validation of these outcomes and mechanistic investigations.*

Keywords: *Inhibitor of DNA-binding, Non-small cell lung cancer, Gene expression, Prognosis*

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INTRODUCTION

Non-small cell lung cancer (NSCLC), primarily comprises of adenocarcinoma (AC) and squamous cell carcinoma (SCC) [1]. Despite advancements in multidisciplinary treatment modalities, prognosis remains unfavorable, particularly in advanced stages, revealing the critical need for novel prognostic markers. Inhibitors of DNA-binding/differentiation (ID) family proteins regulates DNA binding by inhibiting bHLH proteins and are implicated in various cellular functions and tumorigenesis [2].

Prognostic assessment of IDs expression has been conducted in SCLC [3], but exploration in NSCLC, particularly at the mRNA level of individual ID, is limited. Inhibitor of DNA-binding

1 overexpression worsens lung AC outcomes [4] but improves prognosis in brain and breast cancer [5]. Elevated ID2 is linked to poor survival in breast cancer and neuroblastoma [6]. Abnormal ID2/ID3 expression affect breast cancer cell proliferation, while increased ID3 suppresses proliferation and increases apoptosis in cisplantin-resistant lung adenocarcinoma cells [7]. Inhibitor of DNA-binding 4 overexpression induces cisplatin resistance and apoptosis inhibition through activation of phospho-p38 MAPK signaling in A549 cells [8].

This study investigated the correlation between IDs gene expression and prognosis in NSCLC patients using multiple public databases. Furthermore, the association between IDs expression and clinicopathological features was analyzed.

METHODS

Oncomine analysis

Inhibitors of DNA-bindings expression in normal vs NSCLC samples were compared with unpaired t-tests using the Oncomine database, with a fold change > 2. P < 0.01 was considered statistically significant.

UALCAN database

An unpaired *t*-test compared ID expression between normal and tumor tissues using The Cancer Genome Atlas (TCGA) NSCLC dataset from UALCAN database. *P* < 0.01 was considered statistically significant.

Survival analysis

The Kaplan-Meier plotter [9] assessed IDs correlation with overall survival in NSCLC patients using data from 1,926 lung cancer patients. Patients were grouped by IDs median mRNA expression value. Hazard ratios, 95 % confidence intervals, and *p*-values were calculated. Forest plots were generated using Graph Pad Prism software, excluding potentially biased arrays. *P* < 0.05 was considered statistically significant. Representative affymetrix IDs for each ID gene in NSCLC are presented in Table 1.

Also, 994 NSCLC patients (500 adenocarcinoma, 494 squamous cell carcinoma) RNA-seq data and survival information were downloaded from TCGA. Patients were divided by ID mRNA median into high and low-expression groups. Kaplan-Meier plots evaluated the correlation with 5-year overall survival (*p* < 0.05). GraphPad Prism plotted survival curves, with no patient overlap between platforms.

Table 1: The Affymetrix ID of ID genes used in this study

ID family	Affymetrix ID
inhibitor of DNA-binding 1	208937_s_at
inhibitor of DNA-binding 2	201565_at
inhibitor of DNA-binding 3	207826_at
inhibitor of DNA-binding 4	209292_at

RESULTS

Expression levels of ID members in NSCLC

mRNA expression differences of four IDs between tumor and normal samples across various cancers using the Oncomine database were identified (Figure 1). A total of 445, 457, 420, and 442 unique analyses accepted for ID1, ID2, ID3, and ID4, respectively. In lung cancer, ID1, ID2, ID3, and ID4 proteins exhibited significantly higher expression in normal tissues compared to tumor tissues across multiple studies. This trend persisted consistently in lung cancer compared to normal tissues (Table 2).

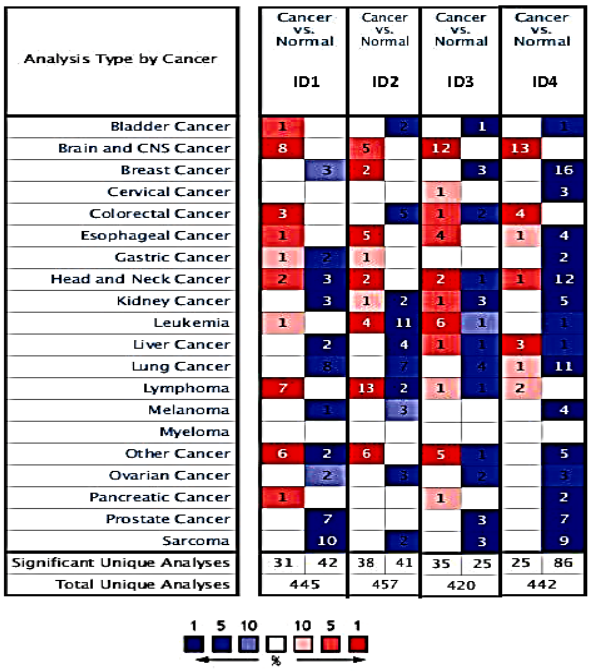

Figure 1: The plot shows mRNA expression patterns of ID members in different human cancers versus normal tissues. Red indicated significant overexpression; blue indicated downregulation. Each cell shows number of analyses meeting the criteria. Color reflects ID gene rank percentile, with fold-change > 2. P < 0.01 was considered statistically significant

Table 2: Number of datasets significantly correlated with ID up-regulation and down-regulation in NSCLC compared to normal samples. *P* < 0.05 was considered statistically significant, fold change 1.5, gene rank: top 10 %

ID family	Number of datasets with up-regulation	Number of datasets with down-regulation
ID1	0	8
ID2	0	11
ID3	0	4
ID4	1	11

Number of datasets with up and down-regulated IDs represented “the number of datasets that significantly correlated with inhibitor of DNA-binding family genes up-regulation and down-regulation”.

In lung adenocarcinoma, ID1 mRNA expression was significantly lower compared to normal tissues (Figure 2 A). In lung squamous cell carcinoma, ID1 expression did not show significant differences (p = 0.39; Figure 3 A), while ID2, ID3, and ID4 exhibited significantly decreased expression compared to normal tissue (Figure 2 B – D; Figure 3 B - D).

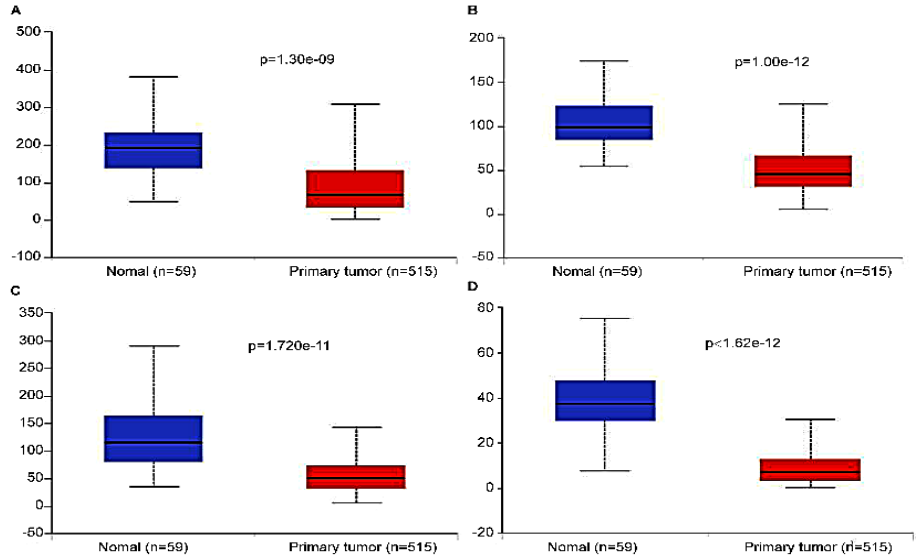

Figure 2: Expression of ID members in normal and primary tumor tissue of lung adenocarcinoma patients. A: ID1, B: ID2, C: ID3, D: ID4

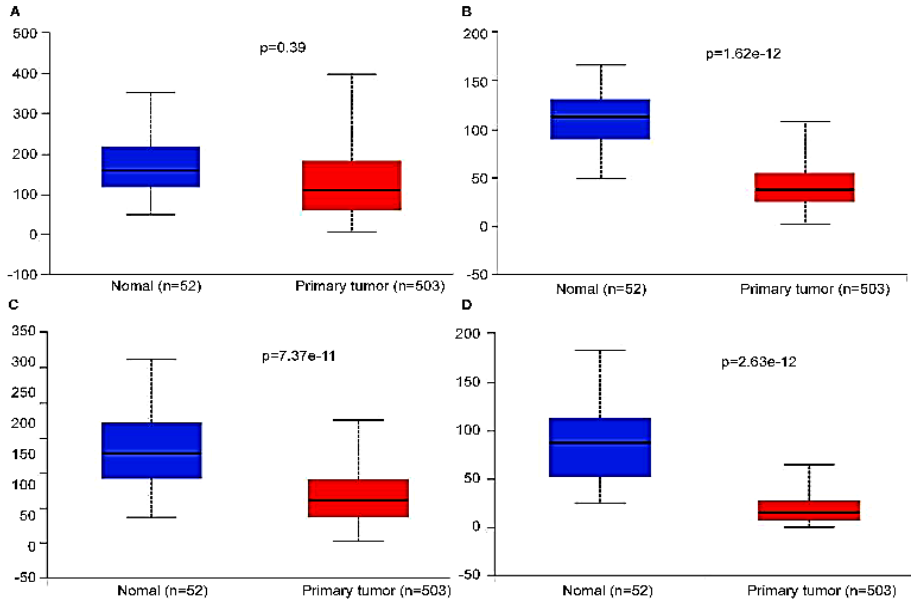

Figure 3: Expression of ID members in normal & primary tumor tissues of lung squamous cell carcinoma patients. A: ID1, B: ID2, C: ID3, D: ID4

Prognostic associations of ID members in all NSCLC patients

ID1 and ID4 showed significant associations with 10-year overall survival (Figure 4). Increased ID1 mRNA expression correlated significantly with poorer overall survival (HR = 1.37; 95 % CI: 1.20 - 1.56; *p* = 1.70e-06; Figure 4 A), while elevated ID4 mRNA expression predicted better prognosis (HR = 0.71; 95 % CI: 0.63 - 0.81; *p* = 2.40e-07; Figure 4 D) in NSCLC patients. However, there was no significant associations between mRNA expression of ID2 (HR = 1.05; 95 % CI: 0.92 - 1.19; *p* = 0.46; Figure 4 B) and ID3 (HR = 1.01; 95 % CI: 0.89 - 1.15; *p* = 0.89; Figure 4 C) and NSCLC patient overall survival.

Furthermore, in 994 NSCLC cases from TCGA, increased ID1 mRNA significantly correlated with reduced 5-year survival (p = 1.30e-03; Figure 5 A), while high ID2 expression was significantly linked to better survival (p = 0.0367; Figure 5 B). There were no significant associations for ID3 (p = 0.1832; Figure 5 C) or ID4 (p = 0.7355; Figure 3 D) expression levels.

Prognostic associations of ID members in NSCLC subtypes

In 720 AC cases, higher ID1 mRNA correlated with poorer prognosis (HR = 1.84; 95 % CI: 1.44 - 2.35; *p* = 7.90e-07; Figure 6 A), while increased ID4 mRNA predicted better prognosis (HR = 0.44; 95 % CI: 0.35 - 0.56; *p* = 2.10e-11; Figure 6 D), consistent with overall NSCLC observations. Also, ID2 and ID3 mRNA weren't linked to AC survival (Figure 6 B - C).

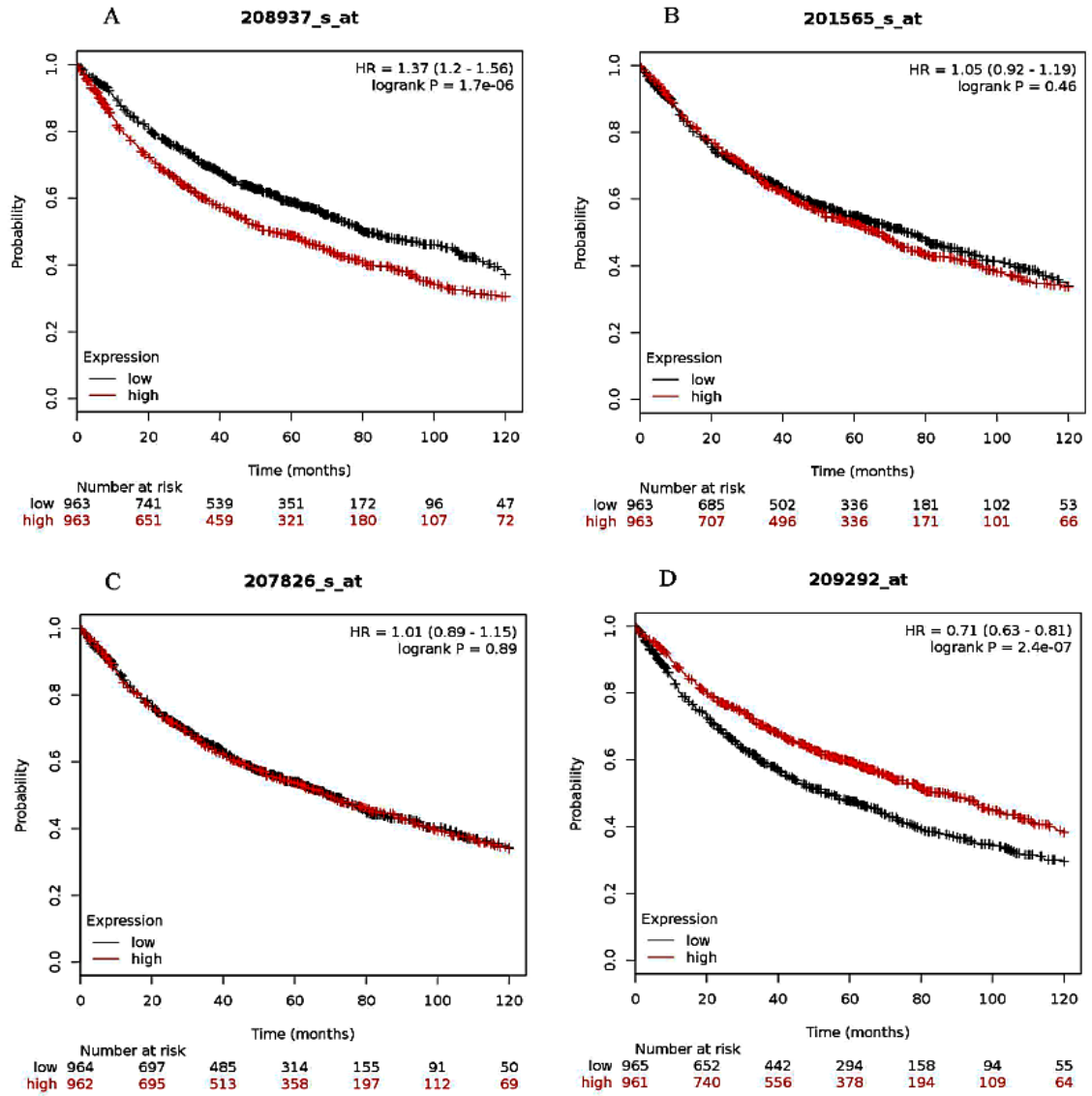

Figure 4: The 10-year overall survival of IDs in all NSCLC patients (n = 1,926): A: ID1, B: ID2, C: ID3, D: ID4

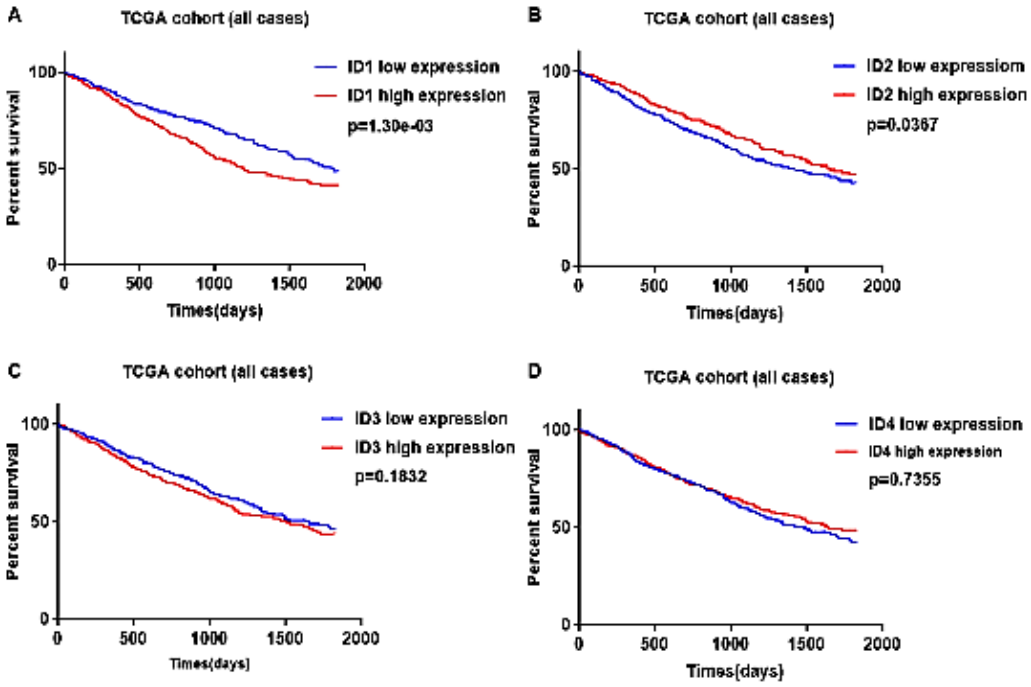

Figure 5: The 5-year overall survival of IDs in TCGA NSCLC dataset (n = 994). A: ID1, B: ID2, C: ID3, D: ID4

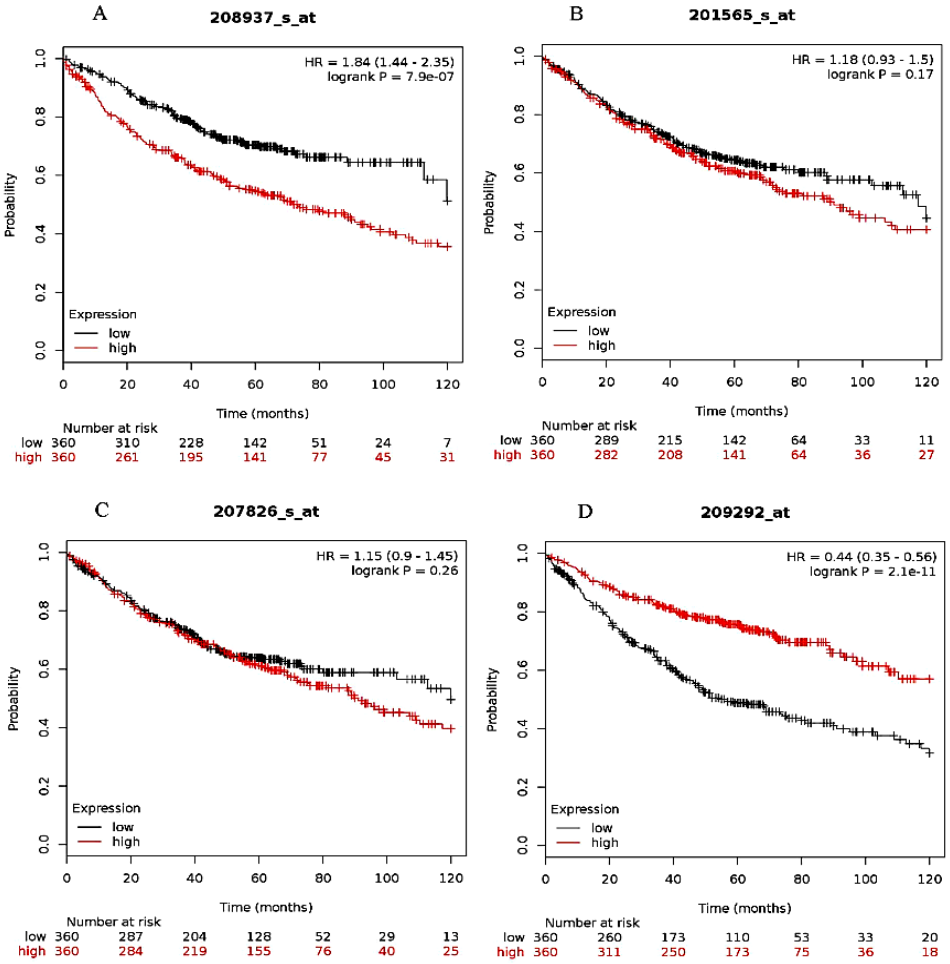

Figure 6: The 10-year overall survival of IDs in lung adenocarcinoma patients (n = 720). A: ID1, B: ID2, C: ID3, D: ID4

Similarly, ID1 mRNA predicted worse 5-year survival in 500 AC cases from TCGA (p = 0.045; Figure 7 A). Furthermore, higher ID2 mRNA was significantly correlated with longer survival in AC NSCLC patients (p = 4.70e-03; Figure 7 B). ID3 (p = 0.3029; Figure 7 C) and ID4 (p = 0.5609; Figure 7 D) mRNA levels showed no significant association with AC patient survival. Elevated ID1 mRNA elevation significantly linked to shorter 10-year survival in SCC patients (HR = 1.37; 95 % CI: 1.08 - 1.74; p = 9.50e-03; Figure 8 A). There was no significant association between mRNA expression of ID members and 5-year survival in TCGA analysis (Figure 9 A - D) as well as ID2 (HR = 0.80; p = 0.069; Figure 8 B), ID3 (HR = 0.92; p = 0.51; Figure 8 C), ID4 (HR = 0.89; p = 0.32; Figure 8 D) mRNA with overall survival.

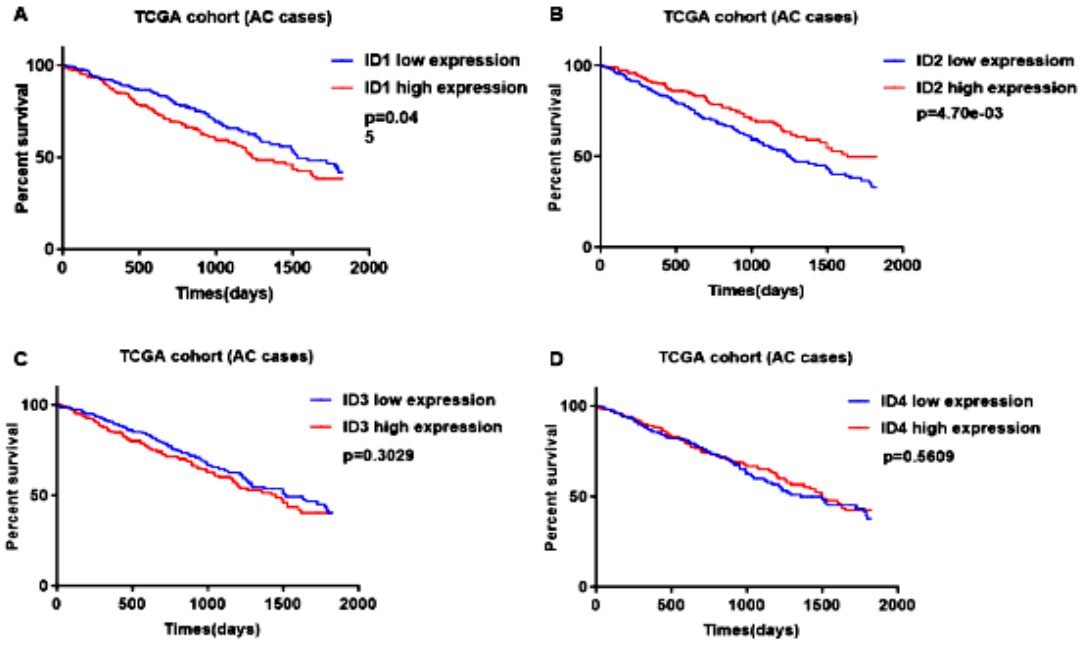

Figure 7: The 5-year overall survival of IDs in lung adenocarcinoma patients (n = 500) A: ID1, B: ID2, C: ID3, D: ID4

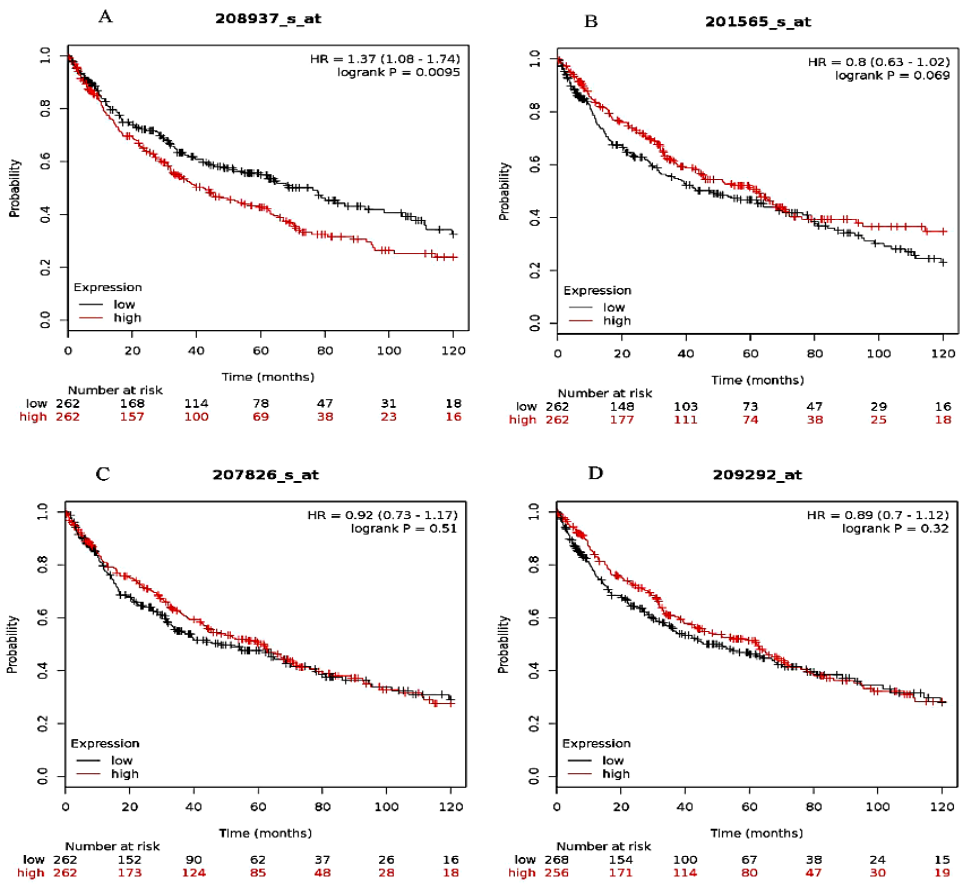

Figure 8: The 10-year overall survival of IDs in lung SCC patients (n=524). A: ID1, B: ID2, C: ID3, D: ID4

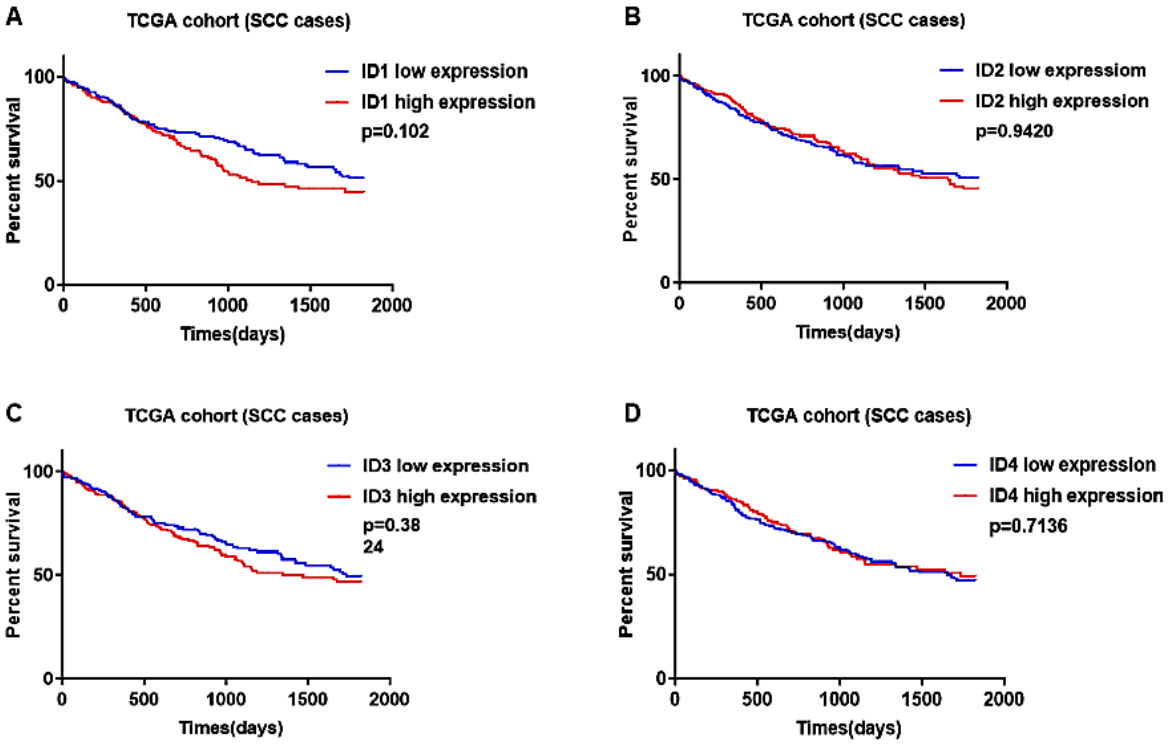

Figure 9: The 5-year overall survival of IDs in lung squamous cell carcinoma patients (n = 494). A: ID1, B: ID2, C: ID3, D: ID4

Prognostic value of ID members in NSCLC patients with different clinicopathological features and treatments

Elevated ID1 mRNA was significantly linked to poorer prognosis in both smokers (HR = 1.58; p = 1.70e-05; Figure 10 A) and non-smokers (HR = 1.81; p = 0.038; Figure 10 B). Higher ID4 mRNA predicted better survival in non-smokers (HR = 0.36; p = 7.80e-04; Figure 10 B), but not in smokers (HR = 0.91; p = 0.37; Figure 10 A). ID1 mRNA correlated with decreased 10-year survival in grade 2 patients (Figure 10 B), while no significant relations were found for ID2, ID3, and ID4 across grades (Figure 11 A - C). ID1 and ID4 were significantly correlated with NSCLC clinical stages (Figure 11). Up-regulation of ID1 (HR = 2.36; p = 2.20e-09; Figure 11 D) and ID2 (HR = 1.38; p = 0.042; Figure 11 D) correlated with shorter survival in stage I, while increased ID4 mRNA was associated with longer survival (HR = 0.39; p = 1.90e-11; Figure 11 D). There were no significant associations in stages 2 and 3 (Figure 11 E - F), likely due to limited patient numbers.

Different mRNA expressions of IDs predicted varied prognoses in patients under different therapies (Figure 12). Higher ID1 (HR = 2.02; p = 3.0e-09; Figure 12 A) and ID2 (HR = 1.30; p = 0.025; Figure 12 A) significantly correlated with shorter survival in surgical resection patients, while increased ID4 was significantly linked to improved survival (HR = 0.56; p = 1.40e-06; Figure 12 A). In chemotherapy-treated NSCLC patients, only higher ID1 expression predicted worse survival (HR = 1.58; p = 0.028; Figure 12 B). There was no significant correlation in patients without chemotherapy (Figure 12 C), with (Figure 12 D) or without radiotherapy (Figure 12 E), likely due to limited database entries.

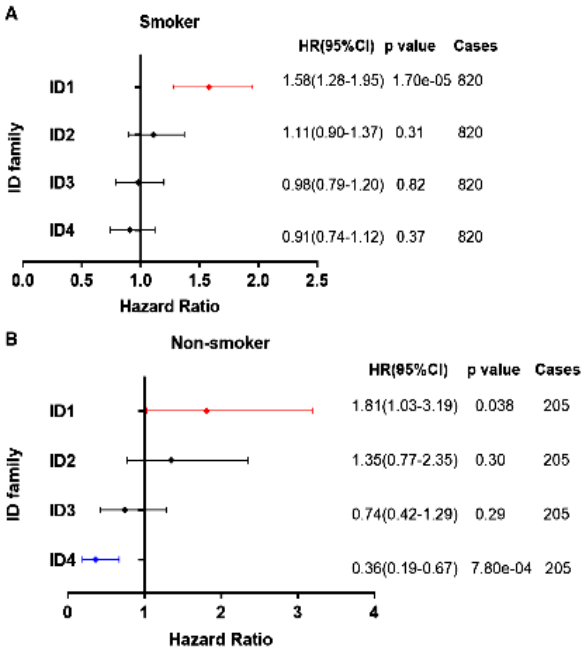

Figure 10: Association between ID members expression and 10-year survival of patients with smoking history. A: Smoking history; B: Non-smoking history

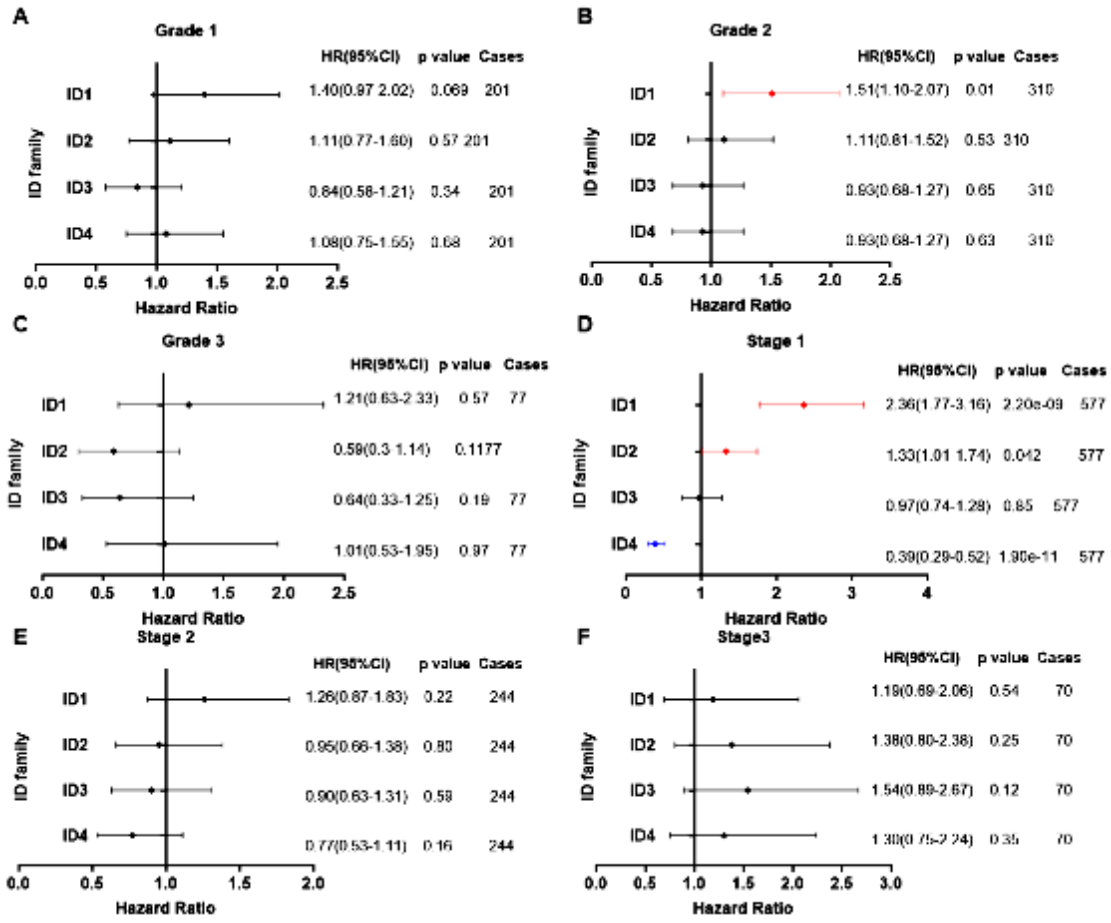

Figure 11: Association between ID members expression and 10-year survival of patients with different grades and stages. A: Grade 1; B: Grade 2; C: Grade 3; D: Stage 1; E: Stage 2; F: Stage 3

DISCUSSION

The ID family members interact with various oncogenic signaling pathways implicated in tumorigenesis [2], making them potential targets for cancer therapy. However, their expression levels and prognostic relevance in NSCLC remains unclear. This study was an integrated analysis of public databases to illustrate the expression of individual IDs in NSCLC. The results revealed distinct mRNA expression patterns between tumor and normal tissues across various malignancies. The ID1 is upregulated in various human cancers [10]. This study showed that increased ID1 expression was significantly linked to poorer overall survival in all NSCLC patients, including AC and SCC patients. Higher ID1 expression often correlates with aggressive features and unfavorable prognosis in multiple tumor types. In NSCLC patients with AC histology receiving chemotherapy at stages I to III, increased ID1 expression was associated with shorter disease-free and overall survival. Conversely, increased ID1 expression predicts better survival in NSCLC patients receiving adjuvant chemotherapy with paclitaxel and cisplatin.

Studies indicated that ID1 expression is associated with clinicopathological features in NSCLC, including histologic type and clinical stages [11]. In patients at stages I to III, decreased cytoplasmic ID1 and ID3 expression predict longer metastasis-free survival, while increased nuclear ID1 and decreased cytoplasmic ID3 expression correlate with higher death risk [12]. This study found that increased ID1 mRNA expression was significantly associated with worse survival in stage I or grade II patients, but not in those with higher stages. Furthermore, elevated ID1 mRNA expression predicted poorer overall survival regardless of tobacco use history. Nicotine, a major component of tobacco, promotes lung tumor progression via nicotinic acetylcholine receptors (nAChR), inducing cell-cycle progression, angiogenesis, and metastasis.

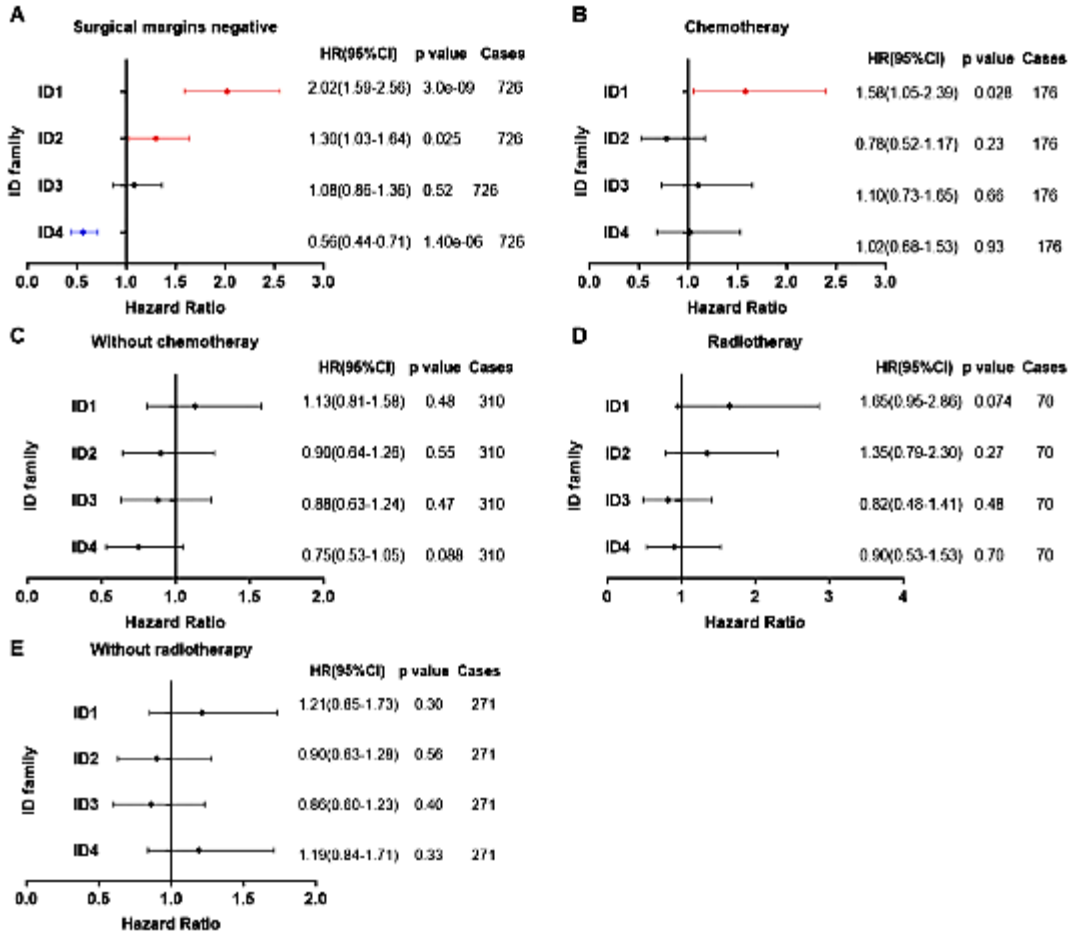

Figure 12: Association between ID members expression and 10-year survival of patients with different treatments. A: Only surgical margins negative, B: Chemotherapy, C: No chemotherapy, D: Radiotherapy, E: No radiotherapy

Depletion of ID1 counteracts nicotine-induced cancer progression. Further research is needed to understand the interaction between ID1 and other ID proteins with nicotine in lung cancer. The ID2 expression is typically low in normal adult cells but upregulated during tumor progression in various cancers, including breast and pulmonary carcinoma [13]. Its prognostic utility in NSCLC (except in SCLC), remains unclear. Increased ID2 expression promotes SCC cell proliferation by upregulating NF-kB and cyclin D1 [14]. Studies suggest that high nuclear ID2 expression correlates with poorer prognosis in poorly differentiated tumors and neuroblastoma [15]. This present study found no significant association between higher ID2 expression and overall survival in all NSCLC patients, but near significance for improved survival in SCC. In contrast, TCGA analysis showed increased ID2 expression predicting better overall survival for lung cancer patients, possibly due to detection method differences. High ID2 expression predicted shorter survival in stage I cancer patients. There was no correlation between ID2 expression and survival regarding smoking history or grades, except in patients with negative surgical margins where high ID2 expression correlated with worse overall survival.

Similarly, ID3 is implicated in cell proliferation and disease pathogenesis [16]. Upregulation in lung AC cells inhibits tumor growth and induces apoptosis [17], suggesting a potential therapeutic alternative. However, there was no significant correlation between ID3 expression and NSCLC prognosis or clinical parameters such as tumor stage, grade, smoking status, or treatment history. Studies have explored the role of ID4 in various tumors, citing its association with patient prognosis in lung tumors [18]. In NSCLC, increased ID4 mRNA expression was linked to better prognosis, especially in lung AC patients and those without a smoking history. Moreover, elevated ID4 expression correlated with longer survival in stage I patients and those undergoing surgical resection. However, ID4 expression did not significantly impact survival in patients receiving chemotherapy or radiotherapy, despite

its role in promoting cisplatin resistance and apoptosis induction in NSCLC cells [8].

Limitations of this study

This study has some limitations. Although, this study identified differential ID expression and clinical relevance in NSCLC, the underlying mechanisms were not accounted for. Investigating the therapeutic potential by targeting these IDs and their associated mechanisms in future research is required.

CONCLUSION

In NSCLC, ID family mRNA expression is generally lower compared to normal tissues. High ID1 mRNA predicts worse overall survival, while increased ID4 mRNA correlates with better survival, especially in AC. Furthermore, ID2 and ID3 mRNA expressions shows no significant association with overall survival. There is the need for future validation of these outcomes and mechanistic investigations.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Conceptualization: Denggang Fu, Xin Wang; Methodology: Denggang Fu, Biyu Zhang, Jinghui Sun; Formal analysis: Denggang Fu; Data curation: Biyu Zhang, Denggang Fu; Original draft preparation: Denggang Fu, Biyu Zhang; Review and editing: Denggang Fu, Jueping Feng, Xin Wang: Project administration: Denggang Fu, Xin Wang.

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