Cancer Management and Research

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ORIGINAL RESEARCH

ctDNA *SNORD3F* Hypermethylation is a Prognostic Indicator in EGFR-TKI-Treated Advanced Non-Small Cell Lung Cancer

Bin Liu*, Bingtian Zhao*, Yan Yin, Yan Jiang, Xue Feng, Lei Wang, Liang Zhai, Guangxin Liu, Dongsheng Shi, Jianwen Qin

Department of Respiratory and Critical Medicine, Tianjin Chest Hospital, Tianjin, People's Republic of China

*These authors contributed equally to this work

Correspondence: Dongsheng Shi; Jianwen Qin, Tianjin Chest Hospital, No. 261, Taierzhuang South Road, Jinnan District, Tianjin, 300051, People's Republic of China, Email shi_ds@126.com; qinjianwen2005@aliyun.com; 5020200603@nankai.edu.cn

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Purpose: DNA methylation plays a regulatory role in the oncogenesis and tumor progression and is valuable in the diagnosis and prognosis of cancer. While circulating tumor DNA (ctDNA) is widely used in the detection of oncogenic mutations and the guidance of treatment in advanced non-small cell lung cancer (NSCLC), studies of ctDNA methylation remains insufficient. We aim to investigate the methylation profiles of ctDNA in patients with advanced NSCLC undergoing EGFR-tyrosine kinase inhibitor (EGFR-TKI) therapy and to discover novel biomarkers with predictive or prognostic value.

Patients and Methods: We recruited 49 patients with *EGFR*-mutated advanced NSCLC undergoing EGFR-TKI as first-line treatment. Utilizing next-generation sequencing, we examined the somatic mutations and methylation signatures within the tumor-associated genomic regions of ctDNA from pre-treatment blood. Subsequently, we explored the association of these molecular features with the patients' response to therapy and their progression-free survival (PFS).

Results: Genomic mutation profiling revealed no significant association of PFS or best overall response (BOR) and ctDNA status. Evaluation of ctDNA methylation showed a negative correlation between the methylation of small nucleolar RNA (snoRNA) genes and PFS (R=−0.31, *P*=0.043). Furthermore, high-level methylation of *SNORD3F* was associated with poorer PFS (mPFS 346d vs 243d, HR 0.49, 95% CI 0.24–0.93, *P*=0.029).

Conclusion: Our study explored the prognostic value of ctDNA methylation in patients with advanced NSCLC undergoing targeted therapies and first revealed the predictive role of *SNORD3F*.

Keywords: ctDNA, EGFR, DNA methylation, lung cancer, snoRNA, *SNORD3F*

Introduction

Lung cancer accounts for approximately 18% of global cancer deaths, with non-small cell lung cancer (NSCLC) accounting for 80% to 85% of cases.[1] Epidermal growth factor receptor (*EGFR*) is a key oncogenic driver in NSCLC, with over 40% of patients harboring *EGFR* mutations (mut*EGFR*), particularly prevalent in Asia.[2,3] For advanced NSCLC with classical mut*EGFR* (19del and L858R), EGFR tyrosine kinase inhibitors (EGFR-TKIs) are the standard first-line treatment.[4]

Circulating tumor DNA (ctDNA) analysis has been utilized to identify somatic mutations in advanced NSCLC.[5] The BENEFIT study prospectively demonstrated the feasibility of liquid biopsy in guiding EGFR-TKI treatment, with 188 ctDNA-detectable mut*EGFR* patients showing objective response rate (ORR) and progression-free survival (PFS) consistent with tumor-detectable mut*EGFR* to gefitinib treatment.[6] These findings have been corroborated by additional research,[7–9] highlighting the superior therapeutic responses in patients with classical ctDNA mut*EGFR*,[10] particularly the 19del subtype.[11] Furthermore, the presence of pre-treatment ctDNA could potentially correlate with the survival outcomes for NSCLC patients undergoing targeted therapy. A meta-analysis of 11 studies encompassing 1,626 patients suggested a non-significant trend towards shorter PFS in patients with detectable ctDNA, but heterogeneity and

Received: 19 April 2024
Accepted: 27 August 2024
Published: 11 October 2024

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publication bias were obvious.[12] This indicates a need for further research into predictive biomarkers through liquid biopsy.

DNA methylation involves adding a methyl group to the 5th position of the cytosine ring and usually causes gene silencing.[13] The aberrant ctDNA methylation pattern has been detected in cancer and proposed as a potential biomarker for diagnosis and prognosis.[14,15] Previous studies have indicated that patients with *EGFR* amplification exhibited genome instability and reduced DNA methylation, which may correlate with the response duration to TKI treatment.[16] Furthermore, the methylation status of certain genes may influence the resistance and sensitivity to EGFR-TKIs.[17–19] However, the exact role of DNA methylation in EGFR-TKI treatment remains an area with numerous unanswered questions. The potential of ctDNA methylation to predict therapeutic outcomes or relapse risk is an intriguing field that warrants more clinical investigation.

Small nucleolar RNAs (snoRNAs) are a class of small RNA molecules that play crucial roles in the chemical modifications of other RNAs. Changes in the expression of snoRNA genes in lung cancer have been reported associated with clinical prognosis.[20] snoRNAs can regulate cancer-related pathways, thereby modulating cell proliferation, migration, and invasion. The overexpression of SNORA71A in NSCLC tissues can trigger the MAPK/ERK signaling cascade, identifying it as an oncogenic driver and prognostic indicator in NSCLC.[21] SNORA38B interacts with the E2F transcription factor 1, modulating the GAB2/AKT/mTOR pathway and creating an immunosuppressive microenvironment. Inhibition of SNORA38B can augment the therapeutic effectiveness of immune checkpoint inhibitors.[22] snoRNAs have the potential to serve as diagnostic and prognostic markers, but their roles in disease control have not yet been fully verified.

In this prospective single-center study, we aimed to identify novel ctDNA methylation prognostic markers for advanced NSCLC with mut*EGFR*. We used a target variation-detected next-generation sequencing (NGS) panel and a methylation sequencing panel to test ctDNA from pre-treatment blood samples. Patients with tumor-detectable mut*EGFR* were selected to analyze the correlation between the ctDNA methylation profiles and PFS as well as reponses to EGFR-TKI therapy. For the first time, we revealed the potential predictive role of the methylation level of the snoRNA gene *SNORD3F* in advanced NSCLC undergoing EGFR-TKI treatment.

Material and Methods

Patients

From April 2018 to September 2021, we conducted a prospective screening of all adult patients with untreated lung adenocarcinoma at stages IIIb to IV who visited our department. A total of 85 patients consented to participate and underwent testing. Patients were excluded from the final analysis dataset for the following reasons: lack of tumor tissue testing, no detectable mut*EGFR* or only T790M detected, non-use of EGFR-TKIs as first-line treatment or unclear treatment modalities, incomplete ctDNA methylation testing, presence of other malignancies, blood transfusion within the 3 months prior to testing, or any conditions deemed by the investigators as unsuitable for trial participation. Ultimately, 49 patients met the criteria for the analysis and completed the follow-up.

DNA Isolation from Tumor Tissue and Blood

Genomic DNA was extracted from the tissue specimens using the QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany). The extracted DNA was then quantified using a Qubit 2.0 fluorimeter (Life Technologies, CA, USA). Approximately 10 mL of blood were collected in Streck tubes (DNA preserving tubes) and processed immediately within 72 hours of collection. Whole blood was centrifuged at 2,400×g for 10 min at 4°C. After discarding the red blood cells and buffy coat, we centrifuged the plasma at 16,000×g for 5 min at 4°C. The supernatants were subsequently stored at −80°C until further assays. cfDNA from plasma was purified using a QIAamp Circulating Nucleic Acid Kit, according to the manufacturer's instructions.

Capture-Based Targeted DNA Sequencing

The genomic profiles of cfDNA samples were assessed by performing capture-based targeted deep sequencing which covers the whole exons of 168 cancer-related genes. The DNA quality and size were assessed by a high-sensitivity DNA

assay using a bioanalyzer. All indexed samples were sequenced using the NextSeq 500 system (Illumina, Inc). with paired-end reads.

Bisulfite Targeted Sequencing and Methylation Data Processing

DNA was sequenced using an enhanced linear-splinter amplification sequencing (ELSA-seq) method as described previously.[17] Extracted DNA was firstly converted to single-strand DNA molecules by sodium bisulfite (EZ-96 DNA Methylation-LightningTM MagPrep, Zymo Research), which was ligated to a splinted adapter and performed in the presence of extension primers. Uracil-tolerating DNA polymerase was used to generate whole-genome bisulfite sequencing (BS-seq) libraries. Target enrichment was addressed by lung-cancer-specific methylation profiling RNA baits, and further quantified by real-time PCR (Kapa Biosciences, Wilmington, MA, USA) and sequenced on a NovaSeq 6000 (Illumina, San Diego, CA, USA) using 2×150 bp cycles.

BS-seq data was further analyzed using an optimized pipeline. Raw data was trimmed by Trimmomatic (v.0.32), and then aligned by BWA-meth (v.0.2.2). After alignment, PCR duplicates were marked with Samblaster (v.0.1.20). The low mapping quality (MAPQ <20) or improper pairing reads were cleared by Sambamba (v.0.4.7) for further analyses. The overlapping reads were removed by in-house scripts to avoid double-counting of methylation signals.

Statistical Analysis

Statistical analysis was performed using R version 4.1.0. Fisher's exact test and non-parametric tests were used to compare categorical data, and the Mann–Whitney test was used to analyse differences in continuous variables between groups. Correlation analysis was conducted using the Pearson's or Spearman's method. Survival analysis was performed using Log rank test. Multivariate Cox regression analyses were conducted to assess the efficacy of factors as prognostic indicators. P values<0.05 were considered to indicate statistical significance.

Ethics Approval and Informed Consent

The study was conducted following the Declaration of Helsinki, and was approved by the Medical Ethics Committee of Tianjin Chest Hospital (No. 2020YS-038-01). Informed consent was obtained from all subjects involved in the study. Written informed consent was obtained from the patients for the publication this paper.

Results

Patient Characters and Clinical Features Associated with Therapeutic Efficiency

From April 2018 to September 2021, 85 Chinese NSCLC patients were enrolled in this study, with data from 49 patients harboring *EGFR* mutations ultimately subjected to final analysis (Supplementary Table 1). All the patients were diagnosed with adenocarcinoma at a median age of 69 years old (IQR 61–75), and most of them (48/49) were in stage IV. The numbers of males and females in the cohort were comparable (27 females and 22 males). 28.6% (14/49) had a smoking history. 61.2% (30/49) of patients did not take surgeries. All patients underwent TKI therapy as 1st line treatment, and 6 of them also synchronously or sequentially received anti-vascular treatment, chemotherapy, or immunotherapy. 77.6% (38/49) got a partial response (PR) as the best response for the first-line treatment, 8.2% (4/49) and 10.2% (5/49) got stable disease (SD) and progressive disease (PD) respectively (Table 1). Till the cutoff date, 44 patients relapsed, and the median progression-free survival (PFS) was 287 days (IQR 195.25–400).

A suite of analysis, encompassing group comparison, correlation analysis, and survival analysis, was performed to study the association of clinical characteristics and therapeutic efficacy. No significant association between clinical characteristics and best overall response (BOR) was observed, even for sex and smoking history which were previously reported to potentially affect the effect of TKI treatment (Figure 1A). Moreover, female patients showed a worse PFS (P=0.031, HR=0.51, Figure 1B). Multiple variants analysis revealed the significance of sex and smoking history as independent risk factors (Figure 1B). When we correlated BOR to PFS, PR patients did not demonstrate a significantly better PFS than SD/PD patients (P=0.331, HR=0.64), which could be attributed to the limited number of SD/PD patients (Figure 1C).

Table 1 Clinical Characteristics of Enrolled NSCLC Patients

	Overall (n=49)
PFS	
Mean (std)	344.48 (241.62)
Median [IQR]	286.50 [195.25, 400.00]
PFS status	
1	44 (89.8)
NA	5 (10.2)
Age	
Mean (std)	67.63 (9.54)
Median [IQR]	69.00 [61.00, 75.00]
Sex	
Female	27 (55.1)
Male	22 (44.9)
TNM Stage	
IIIC	1 (2.0)
IVA	37 (75.5)
IVB	11 (22.4)
Smoking	
No	35 (71.4)
Yes	14 (28.6)
Histology	
Adenocarcinoma	49 (100.0)
1st line treatment (Line1Type)	
TKI	43 (87.8)
TKI + anti-angiogenesis	2 (4.1)
TKI + chemotherapy	3 (6.1)
TKI + ICI	1 (2.0)
Best of response to 1st line treatment (Line1BR)	
PR	38 (77.6)
SD	4 (8.2)
PD	5 (10.2)
NA	2 (4.1)
Classical *EGFR* mutation or not (EGFR mutation_type_2)	
Classical	44 (89.8)
Non-classical	5 (10.2)
***EGFR* 19del/L858R/non-classical mutation (EGFR_mutation_type_3)**	
19del	20 (40.8)
L858R	24 (49.0)
non-classical	5 (10.2)

Abbreviations: BOR, best overall response; BS-seq, bisulfite sequencing; cfDNA, cell-free DNA; CI, confidence interval; ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor; ELSA-seq, enhanced linear-splinter amplification sequencing; HR, hazard ratio; ICI, immune checkpoint inhibitor; IQR, interquartile range; ncRNA, non-coding RNA; NSCLC, non-small cell lung cancer; ORR, objective response rate; PFS, progress disease survival; PD, progressive disease; PR, partial response; SD, stable disease; snoRNA, small nucleolar RNA; snRNA, small nuclear RNA; std, standard deviation; TKI, tyrosine kinase inhibitor.

Mutational Characteristics of Tumors Were Not Effective Prognostic Biomarkers

Genomic profiling by targeted NGS panels on tumor tissues was performed. The top frequent mutations and clinical features are summarized in Figure 2. Besides *EGFR*, mutations in genes such as *TP53, APC, PIK3CA, RB1, CTNNB1*, and *SMAD4* were commonly observed in this cohort. These genes were also reported as the most frequently mutated in the Chinese NSCLC population. Missense caused by single nucleotide variants was the predominant type identified.

In addition, 89.8% (44/49) of the patients carried classical *EGFR* mutants. *EGFR* 19del and *EGFR* L858R took 40.8% (20/49) and 49% (24/49), respectively (Table 1). We did not observe the difference in PFS between patients harboring

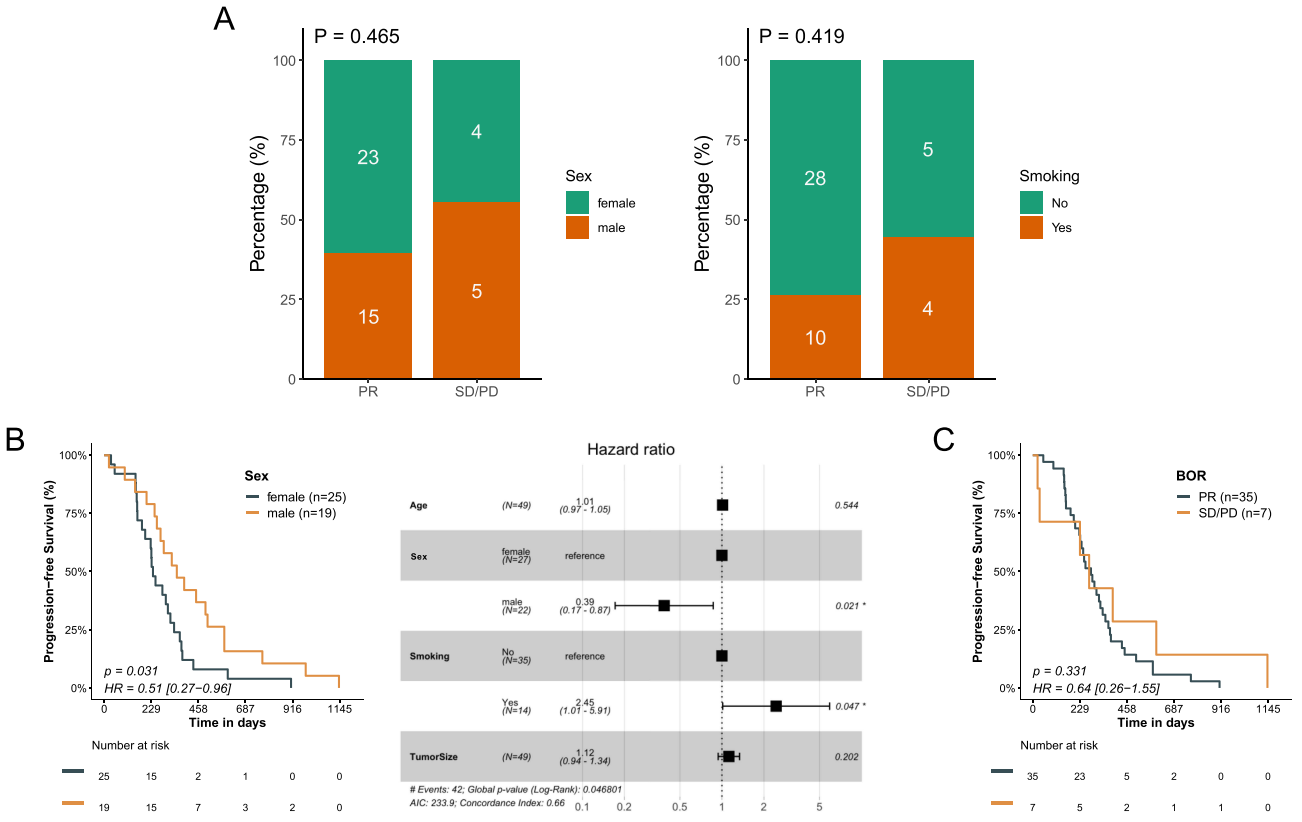

Figure 1 Clinical features of sex and smoking history and their association with the first-line therapeutic efficiency and prognosis. (**A**) Distribution of sex/smoking history in partial response (PR) and stable disease (SD)/progressive disease (PD) patients. (**B**) Kaplan-Meier plots of PFS in patients stratified by sex (left panel) and multivariate regression analysis of clinical features with progression-free survival (PFS, right panel). (**C**) Kaplan-Meier plots of PFS in patients stratified by best overall response (BOR) to first-line treatments.

classical *EGFR* mutations and those with non-classical *EGFR* mutations. For therapeutic response, a higher rate of PR was presented in patients with classical *EGFR* mutations than those with non-classical *EGFR* mutations (P=0.019, Figure 3A). Within the classical mut*EGFR* patients, no association was observed between PFS and therapeutic response with *EGFR* 19del vs *EGFR* L858R (Figure 3B).

The prognostic role of *TP53* co-mutations in advanced *EGFR*-mutated lung adenocarcinomas has been the subject of extensive research. While several studies reported *TP53* co-mutations as a poor prognosis biomarker, others showed no notable impact on PFS. We also examined the prognostic value of *TP53* mutations in our cohort and failed to identify the association of *TP53* co-mutations with PFS (data not shown).

Detection of ctDNA at Baseline Was Not Significantly Associated with Prognosis

In this cohort, 45 baseline blood samples were collected for ctDNA screening. 40 of them have been followed up for survival analysis, with a median follow-up duration of 278 days. According to the genomic mutation detection, 34 patients were identified as ctDNA positive while 11 patients were identified as ctDNA negative at baseline (Figure 4A, Supplementary Figure 1A). No significant differences in PFS and BOR were observed between these groups (Supplementary Figure 2A).

For the ctDNA-positive patients, over half of the alterations in tissues were also found in plasma. 29/34 patients showed mut*EGFR* in baseline ctDNA, and these *EGFR* mutations were consistent with their tumor tissues (Supplementary Figure 1B). Compared to ctDNA-positive patients, fewer mutations were identified in the tumor tissues of ctDNA-negative patients (Supplementary Figure 2B). Patients harboring mut*EGFR* detected in ctDNA exhibited no

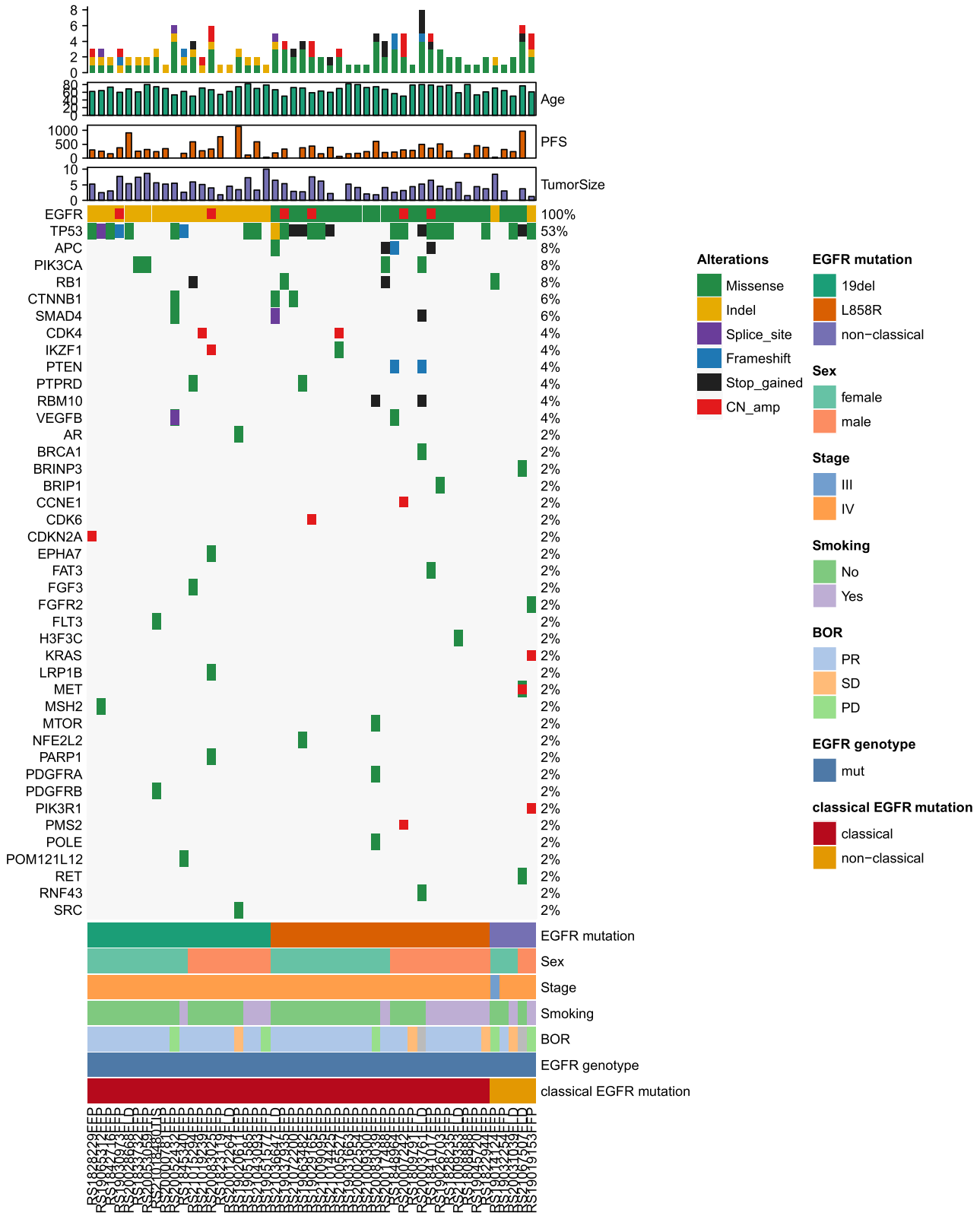

Figure 2 Genetic variation pattern of tumor tissues.

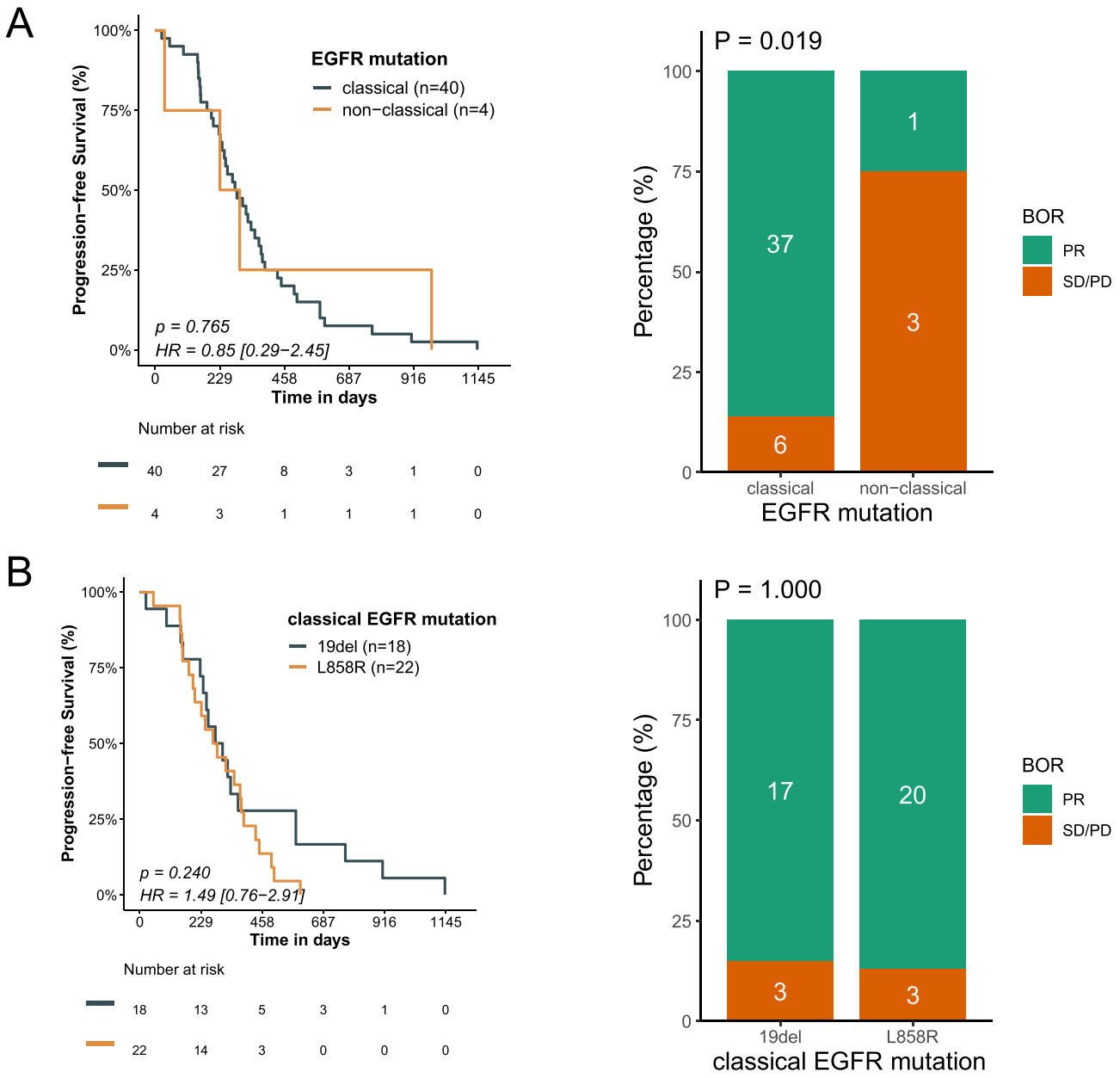

Figure 3 The predictive value and treatment response of *EGFR* mutation in tumor. PFS and best overall response (BOR) in patients stratified by *EGFR* mutation type (**A**) and specifically in patients within classical *EGFR* mutations (**B**).

differences in PFS and BOR to patients with wildtype (Figure 4B, 4C). Since all patients in the cohort had *EGFR* mutations in tumors, it could be attributed to potential false negatives in ctDNA detection.

ctDNA Hypermethylation of snoRNA Genes Was Associated with Poor PFS

Given the inability of baseline ctDNA status to predict the recurrence in our cohort, we further assessed the ctDNA methylation levels covering 8,158 DNA methylation sites and explored their correlation with PFS. We found that the total methylation score showed a tendency to negatively correlate with PFS, but not statistically significant. Our analysis identified two clusters based on total methylation scores, between which no discernible differences in recurrence risk were observed (Figure 5A). Similar results were also observed when examining the top 1,000 and top 500 DNA methylation blocks (Supplementary Figure 3).

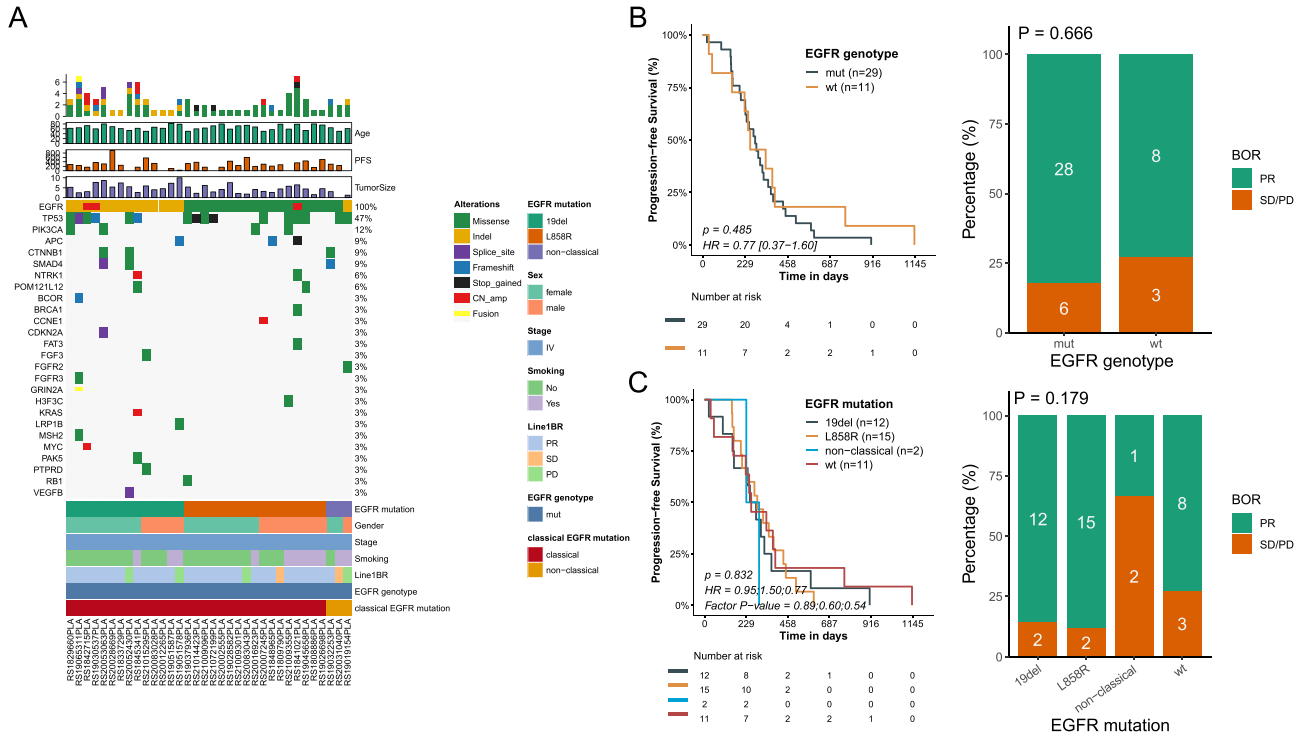

Figure 4 Genetic mutations in ctDNA and the prognostic value of baseline ctDNA. (**A**) Genetic mutation pattern of patients with *EGFR* mutations detected in ctDNA. (**B** and **C**) PFS and BOR in patients stratified by their baseline ctDNA *EGFR* mutation types.

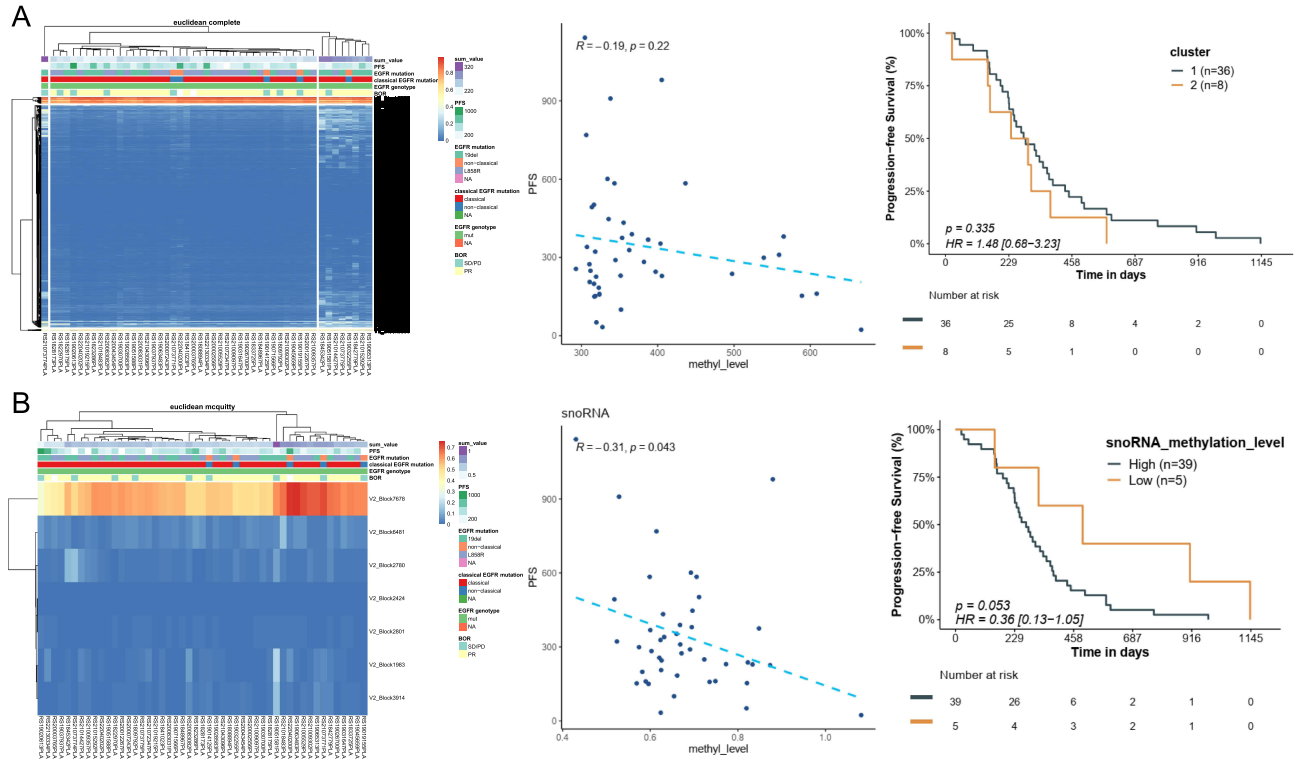

Figure 5 ctDNA methylation levels and their predictive value. (**A**) The overall methylation level of detected ctDNA (left panel) and its correlation with PFS (middle panel), with Kaplan-Meier plots of PFS in patients stratified by their overall methylation level (right panel). (**B**) The methylation level of the snoRNA gene cluster (left panel) and its correlation with PFS (middle panel), with Kaplan-Meier plots of PFS in patients stratified by methylation level of the snoRNA genes (right panel).

The genes within the methylation sequencing panel can be clustered into different types referring to their diverse functions (Supplementary Table 2). To search genes that may impact the prognosis, we probed the relationship between PFS and the methylation levels of each gene cluster, encompassing protein-coding genes, pseudogenes, non-coding RNA (ncRNA) genes, small nuclear RNA (snRNA), and small nucleolar RNA (snoRNA) genes. Only methylation of snoRNA showed significant negative correlations (Figure 5B, Supplementary Figure 4), suggesting that patients with lower methylation levels on snoRNA genes have longer PFS. Multivariate regression analyses also revealed the ctDNA methylation level of snoRNA genes as an independent risk factor to predict the patients' recurrences (Supplementary Figure 5). Although methylation levels of pseudo genes did not show significance on correlation analysis, a pre-set cutoff revealed that reduced methylation level on pseudo genes was associated with poorer PFS (Supplementary Figure 6).

Meanwhile, no differences were observed in methylation levels of different gene types between PR and SD/PD groups, indicating that methylation levels might not directly influence BOR (data not shown).

Hypermethylation of SNORD3F ctDNA Suggested a Poor Prognosis for NSCLC

We further investigated the relationship between the methylation level of individual snoRNA genes and patient prognosis. Heatmap analysis showed that the predominant source of methylation within the snoRNA gene cluster originated from V2_Block7678, encompassing the gene *SNORD3F*. A significant correlation was observed between the methylation level of V2_Block7678 and PFS (Figure 6A). According to this finding, we classified the cohort into high and low methylation groups by the median methylation score of V2_Block7678 in all patients. Patients in the high methylation group exhibited poorer prognosis than those in the low methylation group (P=0.029, HR=0.48, Figure 6B).

Within patients harboring classical *EGFR* mutations, combining mutation types with V2_Block7678 methylation levels could identify the patients with a higher risk of recurrence. Patients with *EGFR* 19del and low-level V2_Block7678 methylation showed the most favorable prognosis, whereas those with *EGFR* 19del and high-level

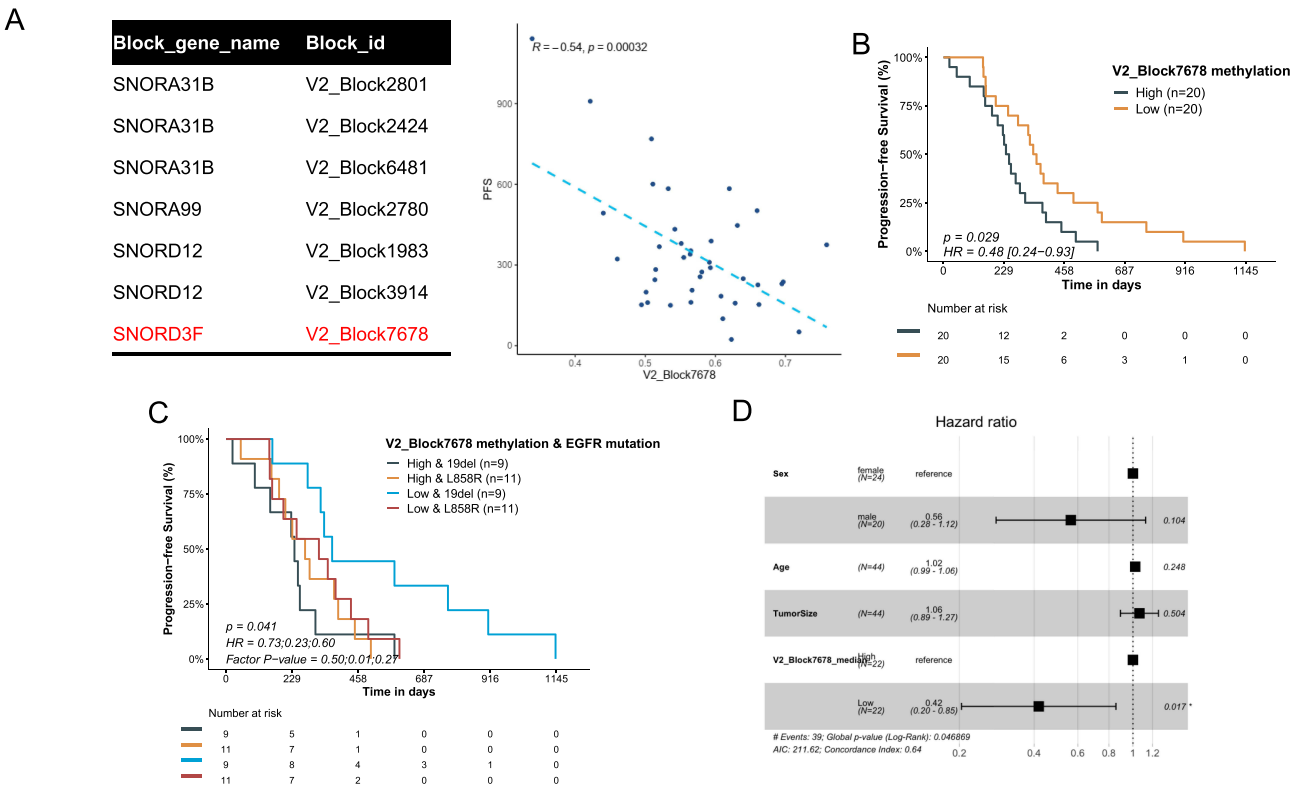

Figure 6 V2_Block7678 (*SNORD3F* included) methylation level and its potential predictive role. (**A**) Gene list of snoRNA genes with corresponding panel blocks (left panel), and the correlation between V2_Block7678 methylation level and PFS (right panel). (**B**) Kaplan-Meier plots of PFS in patients stratified by methylation levels of V2_Block7678 (**B**). (**C**) Kaplan-Meier plots of PFS in patients stratified by methylation levels of V2_Block7678 and *EGFR* mutation types. (**D**) Multivariate regression analysis of clinical features and methylation levels of V2_Block7678.

V2_Block7678 methylation faced the poorest outcomes (Figure 6C). Multivariate regression analyses revealed the methylation level of *SNORD3F* as an independent risk factor to predict the patients' recurrences (Figure 6D).

Discussion

As the recommended first-line treatment for *EGFR*-mutant advanced NSCLC, EGFR-TKIs exhibit variable responses and times to relapse among patients, yet effective predictive biomarkers remain scarce. Our study, based on a Chinese NSCLC patient cohort, introduced innovative ctDNA methylation analysis alongside tissue and ctDNA mutation detection, aiming to identify multi-dimensional biomarkers predictive of EGFR-TKI treatment outcomes.

Differential *EGFR* mutations, such as 19del and L858R, are known to influence the response to targeted therapies. In our study, while classic mut*EGFR* showed better response to targeted treatment, they did not significantly correlate with PFS. The predictive capability of pre-treatment ctDNA status for patient response and prognosis remains a contentious issue, with studies showing contrasting results. For instance, Karllergi et al assessed ctDNA status in 47 mut*EGFR* NSCLC patients who progressed during prior TKI treatment and subsequently underwent Osimertinib treatment, and indicated a significantly shorter median PFS in patients with detectable baseline ctDNA (6.0 months vs 15.9 months, P=0.012).[23] In contrast, in a study that enrolled 99 patients undergoing the first-line TKI treatment, Moiseenko et al found no significant association between baseline ctDNA status and PFS (15.6 months vs 24.1 months, P=0.108).[24] A meta-analysis incorporated data from 11 cohort studies of advanced NSCLC and observed a non-significant trend towards improved PFS of ctDNA-negative patients (pooled hazard ratio [pHR]=1.35, 95% CI 0.83–1.87; P<0.001; I^2=96%).[12] In our study, we did not observe a significant correlation between detectable ctDNA prior to treatment and either response or PFS.

DNA methylation alterations, occurring early in tumorigenesis, has been applied for cancer diagnosis and early detection. Genome-wide DNA methylation signatures have been valuable in predicting recurrence across various cancers.[25–27] Chen et al recently published a ctDNA methylation-based minimal residual disease detection model in resectable NSCLC. They utilized the ELSA-Seq methylation panel and highlighted the heterogeneity between DNA methylation in plasma and tumor.[28] In our study, we used the same technology to explore the predictive value of ctDNA methylation signatures. While the overall methylation score did not yield a significant correlation with PFS, a deeper dive into the methylation profiles of functional gene clusters and individual genes revealed a significant association between PFS and the methylation level of snoRNA genes, especially *SNORD3F*. This finding suggests that the methylation status of certain non-coding RNA genes, such as *SNORD3F*, may serve as a biomarker for predicting treatment outcomes in patients with advanced NSCLC.

Numerous studies has demonstrated the association between the dysregulation of snoRNAs and the genesis and progression of lung cancer, highlighting their potential as prognostic biomarkers.[20–22,29–31] Previous research has indicated that the overexpression of specific snoRNAs might activate cancer-related signaling pathways such as MAPK/ERK (SNORA71A), GAB2/AKT/mTOR (SNORA38B), and PI3K/AKT (SNORA47).[21,22,32] These pathways are downstream of EGFR, suggesting a role for snoRNAs in the biological processes of *EGFR*-mutated tumors. Our study indicates that hypomethylation of *SNORD3F* in ctDNA is associated with improved PFS in patients treated with EGFR-TKIs. This suggests that the expression of SNORD3F may enhance the tumor-suppressive effects of EGFR-TKIs and delay the emergence of resistance. While the biological functions of SNORD3F remain largely unexplored, we hypothesize that it may regulate on pathways involving EGFR and the downstream cascades, thereby influencing the proliferation, migration, and invasion of tumor cells. Our result firstly revealed that the snoRNA gene *SNORD3F* could be a potential prognostic biomarker for advanced NSCLC patients undergoing EGFR-TKI treatment. This inspired the exploration of new prognostic biomarkers and gave a novel insight into the clinical application of ctDNA methylation.

Our study has several limitations that warrant acknowledgment. Firstly, the cohort was small and heterogeneity, which may affect the statistical power for some results and limited the generalizability of our conclusions. Secondly, we did not assess the DNA methylation of paired tissue samples, nor did we quantify the expression of snoRNAs in tissue or plasma. This omission prevents us from confirming the source of methylation aberrations and their actual impact on snoRNA expression. Thirdly, the study did not establish thresholds for patient stratification and lacked an independent validation cohort, which is essential for confirming the reliability and applicability of our findings. Addressing these

limitations will be critical in future research to enhance the validity and applicability of our conclusions and to deepen our understanding of the interplay between DNA methylation and snoRNA expression in the context of cancer biology.

Conclusion

We discovered a significant association between the methylation levels of specific snoRNA genes, particularly *SNORD3F*, and prognosis of advanced NSCLC patient undergoing EGFR-TKI therapy in a Chinese cohort. The hypomethylation of *SNORD3F* in ctDNA was linked to improved progression-free survival, suggesting its potential as a prognostic biomarker. Our results pave the way for further investigation into the clinical utility of ctDNA methylation in personalized medicine approaches for NSCLC.

Data Sharing Statement

All data generated or analyzed during this study are available upon request to the corresponding author.

Institutional Review Board Statement

The study was conducted following the Declaration of Helsinki, and approved by the Medical Ethics Committee of Tianjin Chest Hospital (No. 2020YS-038-01).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study. Written informed consent was obtained from the patients for the publication of this paper.

Acknowledgments

We are grateful for the technical support from Wenbo Dong, Shuo Shi, Bing Li, Qian Li, and Tengfei Zhu.

Funding

This research was funded by the Tianjin Key Medical Discipline (Specialty) Construction Project (TJYXZDXK-049A), and grants from the Tianjin Health Science and Technology Project (NO. ZC20076).

Disclosure

The authors report no conflicts of interest in this work.

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