Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Plasma Exosomal DNA Methylation Profiles and Therapeutic Response in Non-Small Cell Lung Cancer

Minglei Zhang
Binhai County People's Hospital
Fangfang Li
Binhai County People's Hospital
Yang Liu
Wuhan Kindstar Zhenyuan Medical Laboratory Co., Ltd
Bo Hou
Binhai County People's Hospital
Xiaoyan Ji
Binhai County People's Hospital
Suhua Dai
Binhai County People's Hospital
Zhou Gu
Binhai County People's Hospital
Tong Zhou
Wuhan Kindstar Zhenyuan Medical Laboratory Co., Ltd
Lingling Zhao
Wuhan Kindstar Zhenyuan Medical Laboratory Co., Ltd
Xiaodong Yin
yinxd2008@163.com

Binhai County People's Hospital

Research Article

Keywords: non-small cell lung cancer, exosomal DNA, DNA methylation, differentially methylated regions

Posted Date: March 8th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8828378/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Objective

To investigate the differential methylation profiles of plasma exosomal DNA in non-small cell lung cancer (NSCLC) patients, their association with treatment and clinical outcomes, and the epigenetic regulatory mechanisms mediating NSCLC therapeutic response.

Methods

20 NSCLC patients were enrolled, and plasma exosomes were isolated and identified pre- and post-treatment. Bioinformatics analyses were performed to analyze differentially methylated loci (DMLs) and regions (DMRs) of plasma exosomal DNA between pre- and post-treatment samples, and between partial response (PR) and stable disease (SD) groups. Subsequent analyses included DMR genomic features and GO/KEGG enrichment of corresponding differentially methylated genes (DMGs).

Results

Isolated exosomes displayed typical characteristics. Post- vs. pre-treatment comparison identified 3896 DMLs and 43 DMRs (mainly enriched in proximal promoter region), corresponding to key DMGs including KCNJ9, RYR2, INHBB, SOX2, H3C3, H2BC11, RBPJL and MAFA. PR vs. SD comparison found 176 DMLs and 17 DMRs; PR group showed overall hypomethylation, with DMRs enriched in distal intergenic region (clustered on chr16, chr21 and chr22) and only GP1BB as the associated DMG. Treatment-related methylation targeted proximal promoters and distal cis-elements, while efficacy-related epigenetic reprogramming relied on distal regulatory elements. Treatment-associated DMGs mediated cellular stress and signal regulation, and efficacy-associated DMGs were enriched in immune-hematopoietic processes.

Conclusion

Plasma exosomal DNA methylation profiles in NSCLC patients change significantly post-treatment, with PR patients showing characteristic hypomethylation. This epigenetic feature modulates therapeutic response via immune-hematopoietic pathways, and plasma exosomal DNA methylation may serve as a promising epigenetic marker for evaluating NSCLC treatment efficacy.

1 Introduction

Lung cancer ranks among the most prevalent and deadly malignancies globally [1], with non-small cell lung cancer (NSCLC) representing over 85% of all lung cancer cases [2, 3]. Despite ongoing advancements

in surgical resection, chemotherapy, targeted therapy, and immunotherapy, the 5-year survival rate for NSCLC patients remains below 20%. Many patients are diagnosed at an advanced stage, leading to considerable variability in treatment responses and unfavorable prognoses [4]. Currently, tumor node metastasis (TNM) staging system is recognized as an accurate predictor of survival and recurrence in NSCLC [5]. However, this system primarily focuses on tumor size, lymph node involvement, and distant metastasis, which presents significant limitations. It fails to capture the tumor's inherent heterogeneity, the underlying biological characteristics at the molecular level, and individual patient sensitivities to treatment [6]. As a result, there is an urgent need for the identification of non-invasive, precise, and dynamically monitorable molecular biomarkers that can effectively assess treatment responses and prognoses in patients with NSCLC. This advancement is crucial for enhancing clinical outcomes and tailoring treatment strategies to individual patient needs.

Exosomes are a type of nanoscale extracellular vesicles with a diameter ranging from 30–160 nm, widely present in body fluids such as blood and urine. Their core function is to act as a carrier for intercellular communication, carrying and transmitting biologically active molecules such as DNA, RNA, and proteins [7, 8]. These vesicles reflect the physiological and pathological states of the cells of origin and provide stable, biologically valuable diagnostic information. In recent years, exosomal microRNAs have gradually emerged as promising candidates for the development of non-invasive biomarkers [9], whereas research on exosomal DNA has been extremely limited. DNA methylation is the most critical epigenetic regulator of gene transcription, exhibiting distinct DNA methylation profiles between cancer cells and healthy cells [10, 11]. Therefore, methylation analysis can serve as a disease marker, and abnormally methylated DNA encapsulated by exosomes can be released into the circulatory system, providing a novel breakthrough for the non-invasive diagnosis and therapeutic efficacy evaluation of NSCLC. Accordingly, this study aims to explore the differential methylation profiles of exosomal DNA in the plasma of NSCLC patients, analyze their association with therapeutic interventions and clinical outcomes, and reveal the epigenetic regulatory mechanisms that mediate the therapeutic responses of NSCLC. This research will provide novel molecular targets and a theoretical foundation for the non-invasive evaluation of therapeutic efficacy and the formulation of individualized treatment plans for NSCLC.

2 Materials and Methods

2.1 Study subjects

This retrospective study enrolled patients with non-small cell lung cancer (NSCLC) who received chemotherapy or chemoimmunotherapy at Binhai County People's Hospital from January 2024 to October 2025. Plasma samples were collected from all eligible participants at two time points: treatment baseline (prior to the initiation of therapeutic interventions) and immediately after the completion of the full treatment course. Patients were excluded from the study if they had a concurrent diagnosis of other

primary malignant tumors, autoimmune disorders, psychiatric illnesses, poor performance status, or documented hypersensitivity to the therapeutic agents involved in the study.

2.2 Plasma exosomes isolation and characterization

Peripheral blood samples were obtained from all enrolled participants and immediately transferred to EDTA-anticoagulated collection tubes. The samples were subjected to centrifugation at 1900 × g for 15 min at 4 °C to separate plasma fractions, and the resulting supernatant (plasma) was carefully transferred to fresh ultracentrifuge tubes for subsequent exosome isolation. The plasma samples were then centrifuged at 10000 × g for 30 min at 4 °C; the supernatant was discarded, and the resulting pellet was gently resuspended in an appropriate volume of pre-chilled phosphate-buffered saline (PBS, Servicebio, China). An additional round of centrifugation was performed under the same conditions (10000 × g, 30 min, 4 °C), followed by supernatant discarding and resuspension of the final pellet in 100 μL of pre-chilled PBS. The morphological characteristics of the isolated exosomes were visualized using a transmission electron microscope (TEM, HT7700, HITACHI, Japan). Nanoparticle tracking analysis (NTA, Zetaview, Particle Metrix, Germany) was employed to determine the particle size distribution of exosomes. Western blotting was performed to detect the expression of exosome-specific protein markers, including CD9, CD81, and TSG101 .

2.3 Exosomal DNA sequencing and methylation analysis protocol

Exosomal DNA was isolated using a magnetic bead-based DNA extraction kit (IVD5435, Magen Biotech, China). Libraries were constructed with the EpiArt DNA Enzymatic Methylation Kit (EM301, Vazyme, China). Following PCR amplification and magnetic bead purification, qualified libraries were selected for subsequent analysis. High-throughput sequencing was conducted on the Illumina NovaSeq 6000 platform using the paired-end (PE) 150-bp mode. The entire experimental process was performed under sterile and enzyme-free conditions to prevent contamination.

Raw reads were filtered to generate high-quality clean reads. The Bismark[12] software (v0.24.2) was employed for alignment to the human hg38 reference genome. Specifically, Bismark performs C-T and G-A conversions on both sequencing reads and the reference genome, followed by alignment of the converted sequencing data to the reference genomic sequence. Subsequently, uniquely aligned reads were retained, and duplicate reads were removed. Cytosine (C) sites with a sequencing depth of < 10× were filtered out using the methylKit tool [13]. Differentially methylated loci (DMLs) were identified with the criteria of a methylation difference > 20% and p-value < 0.05. Based on the DML results, differentially methylated regions (DMRs) were analyzed using the edmr software [14], with screening conditions set as follows: p-value < 0.05, average methylation difference > 20%, number of CpG sites ≥ 5, and number of DMLs ≥ 3. The ChIPseeker [13] software (v1.38.0) was utilized to annotate genes associated with the

identified DMRs, where the promoter region was defined as the 2000-bp region upstream and downstream of the transcription start site (TSS). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using the clusterProfiler [15] software (v4.10.1).

3 Results

3.1 Patient characteristics

A total of 20 patients with non-small cell lung cancer (NSCLC) were enrolled in this study, including 12 cases of lung adenocarcinoma (LUAD) and 8 cases of lung squamous cell carcinoma (LSCC). The mean age of the patients was 63.25 ± 7.69 years. Based on the TNM staging criteria, the pathological stage distribution was 1 case at Stage I, 3 cases at Stage II, 5 cases at Stage III, and 11 cases at Stage IV. Baseline clinical information and treatment regimens of all patients are summarized in Supplementary Table S1.

3.2 Identification of plasma exosomes

Exosomes were characterized by TEM, NTA and Western blot. TEM analysis demonstrated vesicular structures with prominent refractive properties and a cup-shaped morphology, which are consistent with the typical features of exosomes (Fig. 1A). NTA results indicated that the particle size of exosomes was concentrated at 102.8 nm (Fig. 1B). Western blot assay validated the expression of exosome-specific markers CD9, CD81, and TSG101 (Fig. 1C).

3.3 Differential Methylation Analysis

Based on comprehensive analysis of using the methylKit and edmr tools, we systematically examined the DMLs and DMRs of plasma exosomal DNA, comparing samples collected before and after treatment (pre-treatment vs post-treatment) as well as among different efficacy groups (PR vs SD) (Table 1). In the comparison pre-treatment and post-treatment, we identified a total of 3896 DMLs. Among these, 2280 loci exhibited hypermethylation, indicating a significant increase in methylation levels following treatment, while 1616 loci displayed hypomethylation, reflecting a significant decrease in methylation levels post-treatment. Concurrently, 43 DMRs were identified, including 25 hypermethylated regions and 18 hypomethylated regions (Fig. 2A). Chromosomal distribution analysis revealed a bidirectional regulatory feature of these differential methylation events: chromosomes including chrX, chr22, chr17, and chr3 were predominantly associated with hypermethylated regions, whereas chr20, chr18, and chr8 showed an enrichment of hypomethylated regions (Fig. 2B). These findings indicated that the treatment exerts a broad and chromosome-specific epigenetic regulatory effect on plasma exosomal DNA in patients with NSCLC.

Table 1
Overview of DMLs and DMRs in plasma exosomal DNA of NSCLC patients.

Group	DML-hyper	DML-hypo	DMR-hyper	DMR-hypo
post vs. pre	2280	1616	25	18
PR vs. SD	2	174	0	17

Abbreviations: DML, differentially methylated loci; DMR, differentially methylated region.

In the comparative analysis between different efficacy groups, a total of 176 DMLs were identified. Of these, only 2 displayed hypermethylation status, and the remaining 174 loci were hypomethylated. Meanwhile, 17 DMRs were identified, all of which were hypomethylated regions (Fig. 2C). Chromosomal distribution analysis further confirmed this prominent hypomethylation bias: differential methylation regions of almost all chromosomes were hypomethylated, with only a small amount of hypermethylated regions detected in chr1 and chr19. Notably, chr16, chr21, and chr22 exhibited the highest proportions of hypomethylated regions (with chr16 approaching 25%) (Fig. 2D). These results directly demonstrate that plasma exosomal DNA from patients in the PR group is characterized by global hypomethylation enrichment, and this hypomethylation change aggregates significantly on specific chromosomes, suggesting that epigenetic reprogramming of these chromosomal regions may be closely associated with treatment response.

3.4 DMRs annotation and functional enrichment

To clarify the potential functional effects of DMRs, we further analyzed their distribution patterns across distinct genomic functional regions, as well as the relative distances between their associated transcription factor-binding sites and transcription start sites (TSSs). In the pre- treatment vs. post-treatment comparison, DMRs exhibited a distinct distribution bias across genomic functional regions, with the greatest proportion detected in the proximal promoter region (Promoter ≤1kb, 34.88%), followed by the distal intergenic region (23.6%) (Fig. 3A). Concomitantly, their associated transcription factor-binding sites were predominantly enriched within both the TSS-proximal (0–10kb) and TSS-distal (> 100kb) regions (Fig. 3B), suggesting that treatment-induced methylation alterations may mediate epigenetic regulation by modulating the transcription factor-binding activity at proximal promoters and distal cis-elements. For the comparison between PR vs. SD groups, DMRs showed a more prominent enrichment in the distal intergenic region (41.18%) (Fig. 3C), and the proportion of their associated transcription factor-binding sites located within the TSS-distal (> 100kb) region was markedly elevated (Fig. 3D). The finding suggests that efficacy-related epigenetic reprogramming is predominantly dependent on alterations in distal regulatory elements.

We performed GO functional enrichment analysis (Fig. 4) and KEGG pathway analysis (Table 2) on the differentially methylated genes (DMGs) mapped to DMRs, to delineate the epigenetic regulatory networks driving therapeutic responses and efficacy disparities across molecular function, biological process, and signaling pathway. For the post-treatment vs pre-treatment comparison, GO enrichment

showed that DMGs were primarily engaged in cellular stress and signal regulation processes, including “response to salt”, “regulation of membrane potential”, and “second-messenger-mediated signaling”. At the molecular function level, DMGs were enriched in “ligand-gated monoatomic cation channel activity”, which directly corresponded to the “Circadian entrainment” pathway identified in KEGG analysis. In the PR vs SD group comparison, GO enrichment revealed that DMGs were predominantly involved in immune-hematopoietic processes, such as “myeloid cell differentiation/development” and “positive regulation of platelet activation”. At the molecular function level, DMGs were enriched in “dynein binding”, while cellular component analysis highlighted enrichment in “phagocytic cup”; these results are associated with immune adhesion and invasion inhibition, and are highly consistent with KEGG pathway results, which identified “ECM-receptor interaction”, “Hematopoietic cell lineage”, and “Platelet activation” as key pathways. Collectively, these data indicate that the hypomethylation profile of plasma exosomal DNA in PR patients enhances immune cell infiltration and phagocytic function in the tumor microenvironment, inhibit tumor cell invasion, and ultimately improve treatment response by activating immune-hematopoietic, ECM remodeling, and coagulation-related pathways.

Table 2
KEGG pathway enrichment analysis of DMGs corresponding to DMRs.

Group	Description	p-value	Gene ID
post vs pre	Circadian entrainment	0.0088	KCNJ9/RYR2
	Signaling pathways regulating pluripotency of stem cells	0.0188	INHBB/SOX2
	Systemic lupus erythematosus	0.0188	H3C3/H2BC11
	Oxytocin signaling pathway	0.0214	KCNJ9/RYR2
	Alcoholism	0.0316	H3C3/H2BC11
	Neutrophil extracellular trap formation	0.0332	H3C3/H2BC11
	Viral carcinogenesis	0.0360	RBPJL/H2BC11
	Maturity onset diabetes of the young	0.0379	MAFA
PR vs SD	ECM-receptor interaction	0.0188	GP1BB
	Hematopoietic cell lineage	0.0211	GP1BB
	Platelet activation	0.0265	GP1BB

4 Discussion

This study analyzed the intrinsic connection between the methylation characteristics of plasma exosome DNA in NSCLC patients and therapeutic intervention as well as clinical efficacy, providing a novel epigenetic basis for the efficacy evaluation of NSCLC. The study first confirmed the typical morphological features and specific molecular marker expression of the isolated vesicles through TEM,

NTA and WB verification, laying a solid experimental foundation for the reliability of subsequent methylation analysis. The research data showed that therapeutic intervention could significantly reshape the methylation profile of plasma exosome DNA in NSCLC patients. A total of 3896 DMLs and 43 DMRs were screened out before and after treatment. The differential methylation presented bidirectional regulatory characteristics at the chromosomal level, and treatment-related DMRs were highly enriched in the near-promoter regions. The associated transcription factor binding sites were distributed in the proximal and distal regions of the TSS, suggesting that treatment could achieve epigenetic regulation of downstream genes by regulating the transcription factor binding activity of the promoter and distal cis-elements. Further annotation of the genes corresponding to the differentially methylated regions revealed that treatment-related DMGs included key molecules such as KCNJ9, RYR2, INHBB, SOX2, H3C3, H2BC11, RBPJL and MAFA. Among them, KCNJ9 is a classic potassium ion channel-encoding gene [16], and RYR2 is involved in intracellular calcium ion release and membrane potential homeostasis regulation [17]. The methylation abnormalities of these two genes were highly consistent with the GO-enriched "regulation of membrane potential" and "second-messenger-mediated signalling" processes, as well as the KEGG-enriched "Circadian entrainment" pathway, suggesting that treatment could regulate ion channel function through epigenetic modification, disrupt the circadian rhythm and signal transduction of tumor cells, and thereby inhibit the malignant proliferation of tumor. INHBB and SOX2 are core genes regulating the pluripotency and stemness maintenance of tumor stem cells [18, 19]. The methylation changes of these two genes suggest that treatment can weaken the stem cell characteristics of tumor cells through epigenetic reprogramming, reducing the risk of tumor drug resistance and recurrence. H3C3 and H2BC11 are involved in nucleosome assembly and chromatin structure remodeling [20], their methylation abnormalities are consistent with the DNA damage repair process induced by treatment. RBPJL and MAFA are involved in cell transcriptional regulation and metabolic homeostasis maintenance [21], further expanding the biological coverage of treatment-related epigenetic regulation. In the comparison between different efficacy groups, this study discovered more critical clinical epigenetic features. The plasma exosome DNA of patients in the PR group showed a significant overall hypomethylation-enriched state, with all 17 DMRs being hypomethylated regions. This feature was significantly clustered on chromosomes chr16, chr21 and chr22, and the efficacy-related DMRs were mainly enriched in the distal intergenic regions. This intergroup comparison only identified a single differentially methylated gene, GP1BB, which encodes platelet surface glycoprotein and is a key molecule involved in the ECM-receptor interaction and platelet activation pathways [22]. The hypomethylation modification of GP1BB [23] can simultaneously enhance immune surveillance and promote immune cell infiltration within the tumor microenvironment by regulating platelet activation and the differentiation of hematopoietic cells. Additionally, it can inhibit the invasion and metastatic potential of tumor cells by modulating extracellular matrix adhesion signals. This dual function highlights the core epigenetic mechanism that contributes to improved treatment responses observed in patients within the PR group.

Current studies on epigenetics of NSCLC mainly focus on the methylation profiles of cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA) [24, 25]; however, there remains a significant gap in the

systematic exploration of the relationship between plasma exosomal DNA methylation and treatment response in NSCLC patients. This study fills this gap and presents innovative findings with potential clinical applications. In contrast to the invasive methods associated with tumor tissue puncture and the technical challenges posed by low ctDNA content and high degradability rates in peripheral blood, plasma exosomal DNA benefits from the protective lipid bilayer of exosomes, offering greater stability in body fluids. This stability better meets the dynamic monitoring needs for clinical treatment effects. The research design of this study adopts a dual-dimensional approach, comparing data both before and after treatment as well as between the PR and SD efficacy groups. This design not only elucidates the direct regulatory effects of treatment on the methylation profile but also enables the identification of specific epigenetic modification characteristics closely related to clinical treatment response. Although this study has achieved clinically valuable findings, it is still subject to certain limitations due to its design and experimental conditions. The sample size enrolled is relatively limited, only 20 NSCLC patients, with just 3 cases in the SD group. This limited and unbalanced sample size may affect the stability of statistical analysis results, reduce the statistical power of the conclusion, and may also lead to some potential efficacy-related differential methylation genes failing to be effectively detected. At the same time, this study is a single-center retrospective study, and the participants are all from the same clinical center, inevitably resulting in patient selection bias and thus failing to fully reflect the true characteristics of plasma exosomal DNA methylation in NSCLC patients from different regions, different treatment regimens, and different baseline characteristics. Moreover, this study only conducted the screening and prediction of core differentially methylated genes such as KCNJ9, RYR2, and GP1BB through bioinformatics analysis, and did not conduct in vitro or in vivo experiments to verify the biological functions of these genes or to assess the clinical practicality of the markers through independent clinical cohorts. This limitation somewhat hinders the direct clinical translation of the research conclusions.

Considering the limitations of this study, future research should focus on multiple aspects to enhance the findings and facilitate their clinical application. First, multi-center, prospective clinical trials should be conducted, expanding the clinical sample size and the ensuring a balanced representation of cases in the PR and SD groups. This approach will help minimize sample bias while validating the stability and generalizability of key differentially methylated genes, such as KCNJ9, RYR2, and GP1BB, as well as the related methylated regions identified in this study. Second, it is crucial to select genes closely related to treatment response (e.g., GP1BB) and key regulatory genes (e.g., KCNJ9 and SOX2) to develop NSCLC cell and animal models constructed. Through methylation intervention and gene editing techniques, researchers can thoroughly investigate the specific molecular mechanisms by which core markers regulate tumor cell biological behavior and treatment sensitivity. Finally, integrating the specific methylation modification sites and key genes identified in this study will enable the construction of a plasma exosomal DNA methylation biomarker panel. Thia panel can be used to develop a non-invasive and precise model for evaluating the therapeutic efficacy of NSCLC, incorporating clinical indicators such as TNM staging and traditional tumor markers. Collectively, this study provides preliminarily evidence that plasma exosomal DNA methylation may serve as a potential epigenetic biomarker for

assessing treatment response in NSCLC, providing novel research directions and experimental evidence for precise and individualized diagnosis and treatment of NSCLC.

5 Conclusion

The DNA methylation profile of plasma exosomal DNA in NSCLC patients shows significant changes following therapeutic intervention. Patients in the PR group demonstrate distinct characteristics marked by hypomethylation in their plasma exosomal DNA. This epigenetic feature has the potential to influence the therapeutic response in NSCLC by modulating key signaling pathways, including those involved in immune hematopoiesis and extracellular matrix remodeling. Overall, plasma exosomal DNA methylation appears to be a promising candidate as an epigenetic marker for assessing the therapeutic efficacy of NSCLC treatments.

Declarations

Data availability

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Binhai County People's Hospital (Approval ID: 2024BYKYLL006). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Clinical trial number

not applicable

Funding

This work was supported by the Beijing Kangmeng Charity Foundation (Grant Number: KM-20240715-01).

Consent to publish

Not applicable

References

1. Leiter A, Veluswamy RR, Wisnivesky JP. The global burden of lung cancer: current status and future trends [J]. Nat Rev Clin Oncol. 2023;20(9):624–39. 10.1038/s41571-023-00798-3.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries [J]. CA Cancer J Clin. 2021;71(3):209–49. 10.3322/caac.21660.
3. Osarogiagbon RU, Van Schil P, Giroux DJ, Lim E, Putora PM, Lievens Y, et al. The International Association for the Study of Lung Cancer Lung Cancer Staging Project: Overview of Challenges and Opportunities in Revising the Nodal Classification of Lung Cancer [J]. J Thorac Oncol. 2023;18(4):410–8. 10.1016/j.jtho.2022.12.009.
4. Le Chevalier T. Adjuvant chemotherapy for resectable non-small-cell lung cancer: where is it going? [J]. Ann Oncol. (2010) 21 Suppl 7: vii196-198. 10.1093/annonc/mdq376
5. Kameyama K, Takahashi M, Ohata K, Igai H, Yamashina A, Matsuoka T, et al. Evaluation of the new TNM staging system proposed by the International Association for the Study of Lung Cancer at a single institution [J]. J Thorac Cardiovasc Surg. 2009;137(5):1180–4. 10.1016/j.jtcvs.2008.09.030.
6. Hendriksen BS, Ortiz BA, Wigle DA, Cassivi SD, Saddoughi SA, Puig CA, et al. Risk factors and survival associated with lung cancer recurrence after curative-intent surgery: beyond TNM pathological staging [J]. J Thorac Dis. 2025;17(11):10285–97. 10.21037/jtd-2025-1404.
7. Yu W, Hurley J, Roberts D, Chakrabortty SK, Enderle D, Noerholm M, et al. Exosome-based liquid biopsies in cancer: opportunities and challenges [J]. Ann Oncol. 2021;32(4):466–77. 10.1016/j.annonc.2021.01.074.
8. Li J, Peng J, Guo H, Zheng W, Chen R, Miao C, et al. Exosome-based liquid biopsy for rare and complex diseases [J]. Int J Surg. 2025. 10.1097/js9.0000000000003951.
9. Choudhary A, Poojary SS, Jain P, Chaturvedi H. Das. Identification of novel exosomal miRNAs and their role in diagnosis and prognosis of triple negative breast cancer [J]. BMC Cancer. 2026. 10.1186/s12885-025-15499-6.
10. Thursby SJ, Jin Z, Blum J, Gurau A, Noë M, Scharpf RB, et al. Epigenetic Instability Based Metrics in Cell-Free DNA for Early Cancer Detection [J]. Clin Cancer Res. 2026. 10.1158/1078-0432.Ccr-25-3384.
11. Rodríguez-Paredes M, Feng Y, Gilliam O, Wegner K, Raddatz G, Grönniger E, et al. Non-invasive epidermis sampling for DNA methylation-based prediction of skin cancer phenotypes [J]. NPJ Precis Oncol. 2026. 10.1038/s41698-026-01302-7.
12. Krueger F, Andrews SR. Bioinformatics. 2011;27(11):1571–2. 10.1093/bioinformatics/btr167. Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications [J].
13. Akalin A, Kormaksson M, Li S, Garrett-Bakelman FE, Figueroa ME, Melnick A, et al. methylKit: a comprehensive R package for the analysis of genome-wide DNA methylation profiles [J]. Genome Biol. 2012;13(10):R87. 10.1186/gb-2012-13-10-r87.

14. Li S, Garrett-Bakelman FE, Akalin A, Zumbo P, Levine R, To BL et al. An optimized algorithm for detecting and annotating regional differential methylation [J]. BMC Bioinformatics. (2013) 14 Suppl 5(Suppl 5): S10. 10.1186/1471-2105-14-s5-s10
15. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters [J]. Omics. 2012;16(5):284–7. 10.1089/omi.2011.0118.
16. Goldberg LR, Baskin BM, Beierle JA, Adla Y, Kelliher JC, Yao EJ, et al. Atp1a2 and Kcnj9 Are Candidate Genes Underlying Sensitivity to Oxycodone-Induced Locomotor Activation and Withdrawal-Induced Anxiety-Like Behaviors in C57BL/6 Substrains [J]. Genes Brain Behav. 2025;24(1):e70009. 10.1111/gbb.70009.
17. Noren A, Shiferaw Y. Structural integrity of RyR2 clusters controls cardiac calcium leak [J]. Front Physiol. 2025;16:1731863. 10.3389/fphys.2025.1731863.
18. Liu Y, Zhou Q, Zou G, Zhang W. Inhibin subunit beta B (INHBB): an emerging role in tumor progression [J]. J Physiol Biochem. 2024;80(4):775–93. 10.1007/s13105-024-01041-y.
19. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors [J]. Cell. 2006;126(4):663–76. 10.1016/j.cell.2006.07.024.
20. Tagami H, Ray-Gallet D, Almouzni G, Nakatani Y. Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis [J]. Cell. 2004;116(1):51–61. 10.1016/s0092-8674(03)01064-x.
21. Lv J, Fang M, Sun S, Wang G, Fu S, Sun B, et al. Blockade of the Arid5a/IL-6/STAT3 axis underlies the anti-inflammatory effect of Rbpjl in acute pancreatitis [J]. Cell Biosci. 2022;12(1):95. 10.1186/s13578-022-00819-1.
22. Urbański B, Urbańska Z, Bąbol-Pokora K, Subocz E, Młynarski W, Janczar S. Inherited or Immunological Thrombocytopenia: The Complex Nature of Platelet Disorders in 22q11.2 Deletion Syndrome [J]. Semin Thromb Hemost. 2025;51(8):928–40. 10.1055/s-0044-1801383.
23. Morandi L, Gissi D, Tarsitano A, Asioli S, Gabusi A, Marchetti C, et al. CpG location and methylation level are crucial factors for the early detection of oral squamous cell carcinoma in brushing samples using bisulfite sequencing of a 13-gene panel [J]. Clin Epigenetics. 2017;9:85. 10.1186/s13148-017-0386-7.
24. Hsiao A, Woodward B, Ye P, Varga MG, Altaie G, Lu K, et al. Brief Report: Methylation-Based ctDNA Serial Monitoring Correlates With Immunotherapy Response in NSCLC [J]. Clin Lung Cancer. 2025;26(1):72–7. 10.1016/j.cllc.2024.10.013.
25. Trombetta D, Delcuratolo MD, Fabrizio FP, Delli Muti F, Rossi A, Centonza A et al. Methylation Analyses in Liquid Biopsy of Lung Cancer Patients: A Novel and Intriguing Approach Against Resistance to Target Therapies and Immunotherapies [J]. Cancers (Basel). (2025) 17(18). 10.3390/cancers17183021

Figures

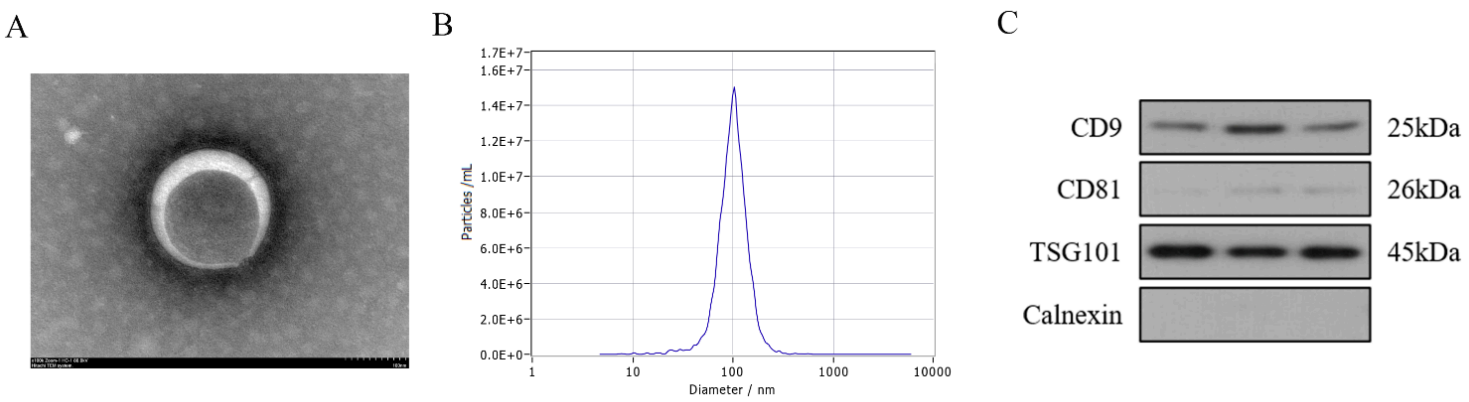


Figure 1

Characterization of plasma-derived exosomes. (**A**) TEM showing the morphology of exosomes; scale bar = 100 nm. (**B**) NTA for measuring the particle size of exosomes. (**C**) Western Blot analysis for detecting exosome-specific marker proteins. TEM, transmission electron microscopy; NTA, nanoparticle tracking analysis.

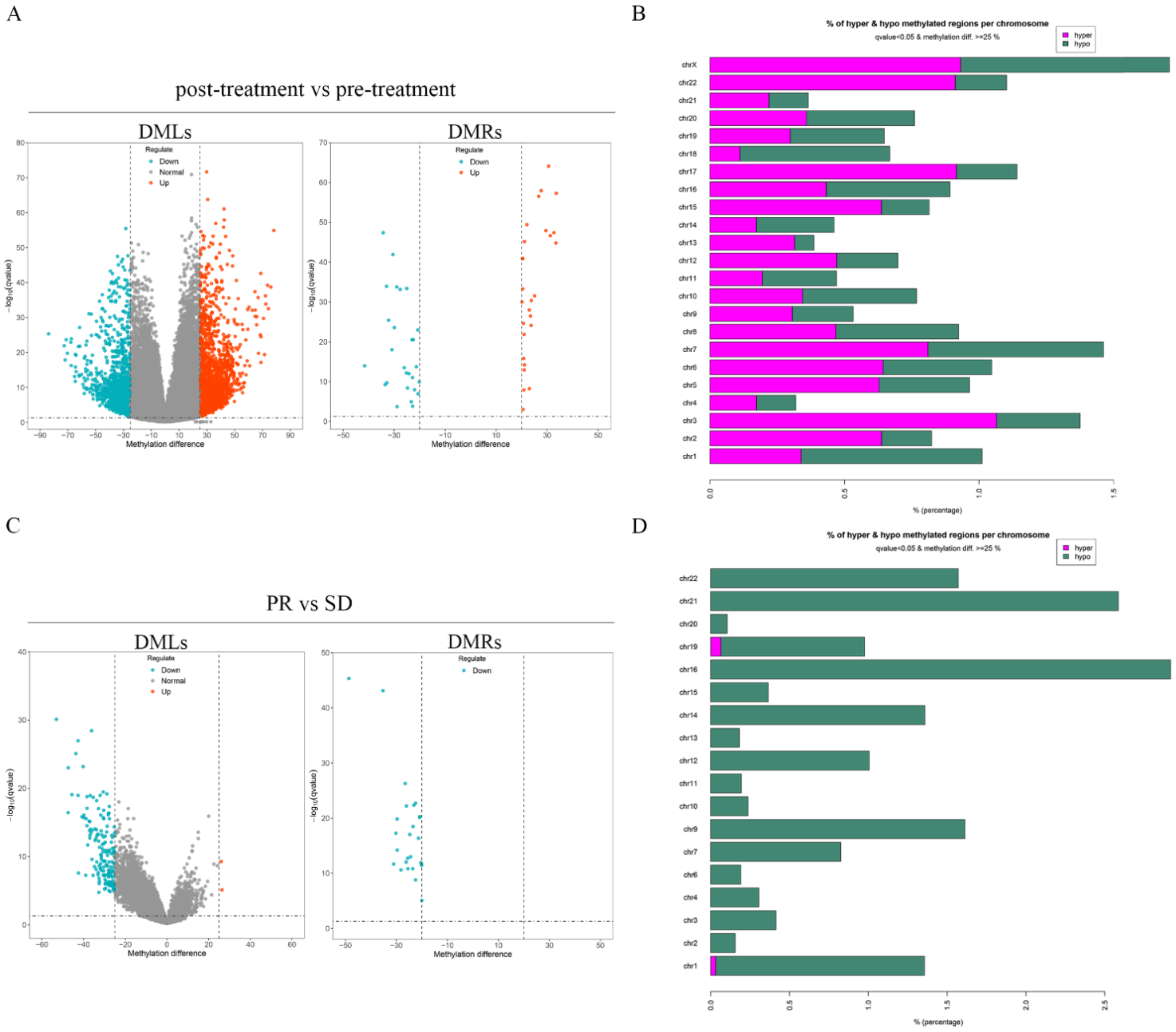


Figure 2

Analysis of differential DNA methylation in plasma exosomes from NSCLC patients. (**A**) Differential methylation profiling of exosomal DNA between post-treatment and pre-treatment groups. (**B**) Chromosomal distribution of DMRs in pre-treatment vs. post-treatment groups. (**C**) Differential methylation profiling of exosomal DNA between PR and SD groups. (**D**) Chromosomal distribution of DMRs between PR and SD groups.

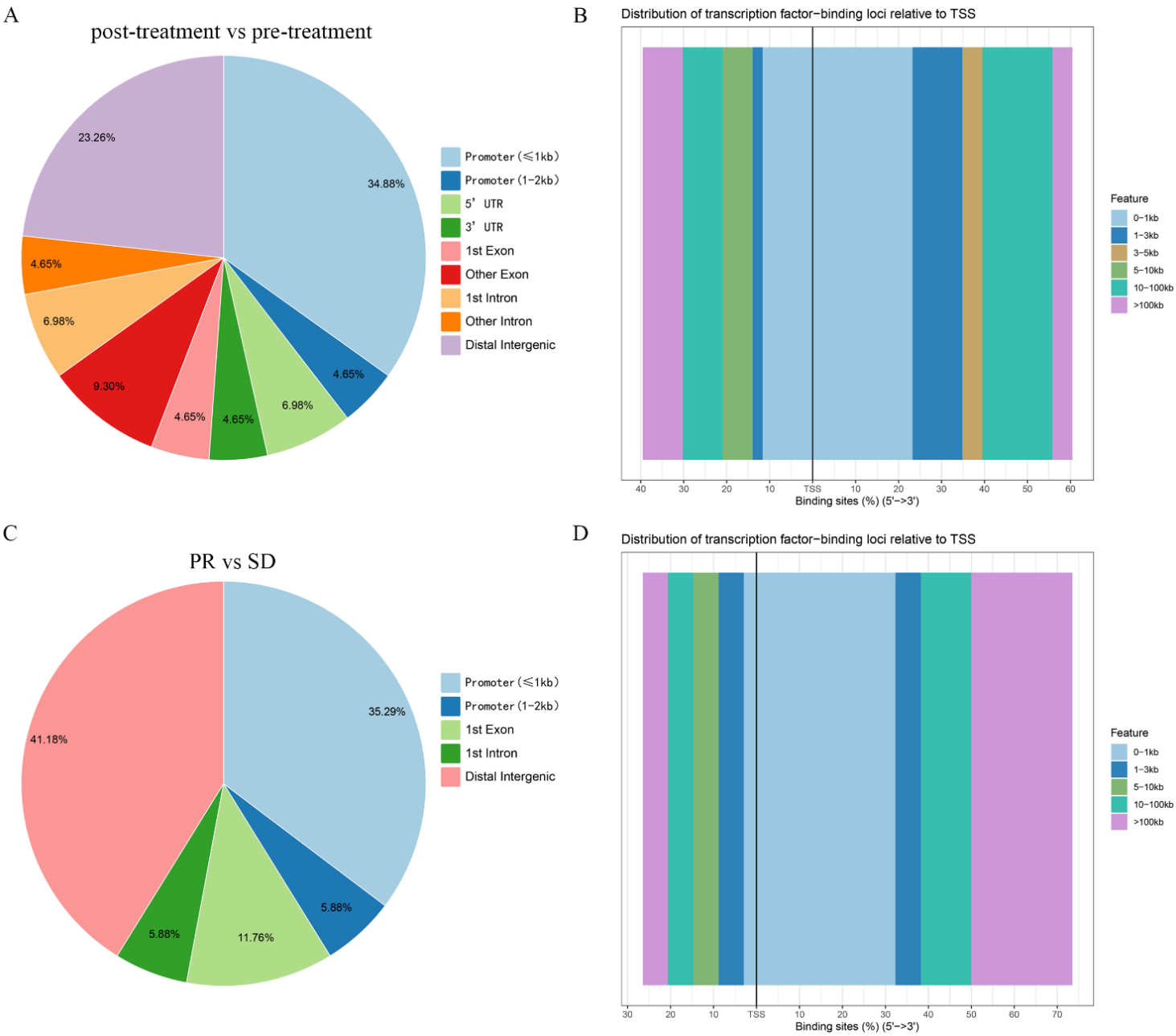


Figure 3

Annotation of DMRs. (**A, C**) Proportional distribution of DMRs across distinct functional regions of the genome. (**B, D**) Distance of DMRs from transcription start sites (TSSs).

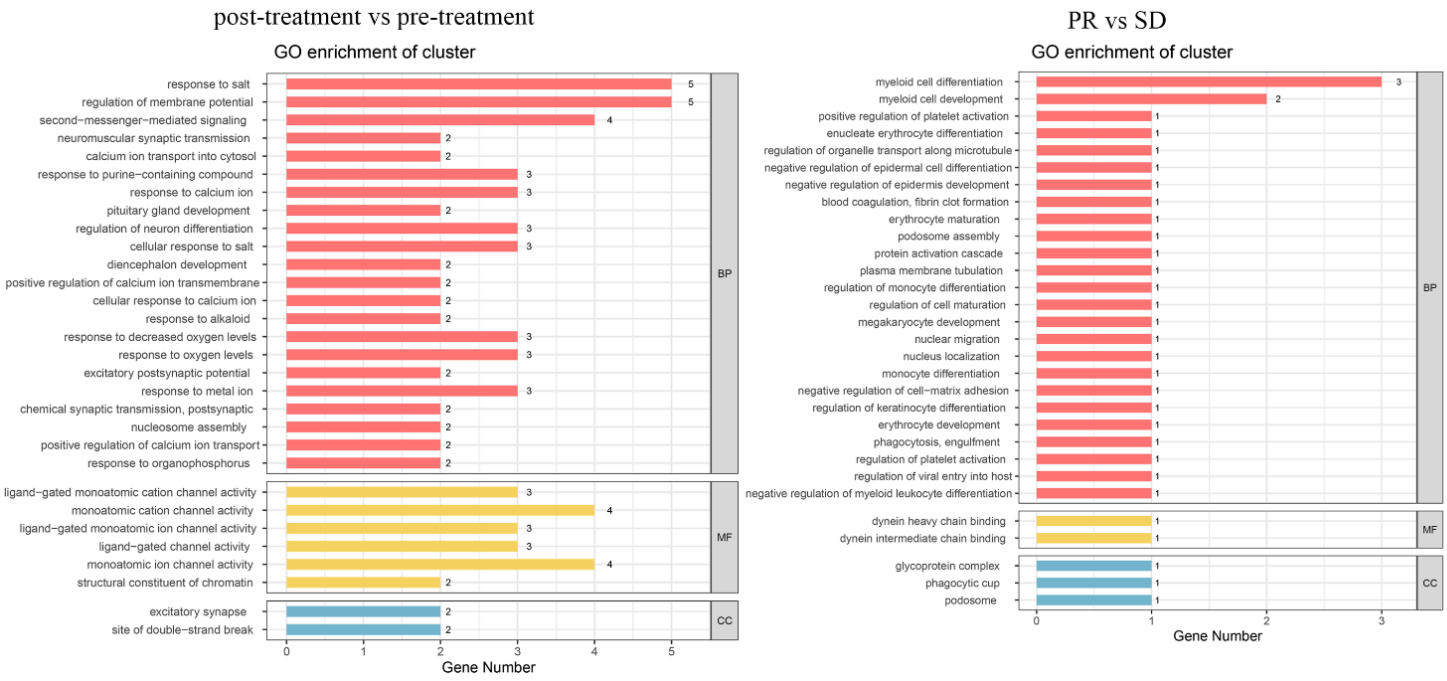


Figure 4

GO functional enrichment analysis of DMGs corresponding to DMRs in post-treatment vs. pre-treatment and PR vs. SD comparisons.