

EP03.04: Metabolic Transcriptomic Subtyping Defines Distinct Immunogenomic and Clinical Landscapes in Non-Small Cell Lung Cancer

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INTRODUCTION

- Over the past decade, antitumor immunotherapies, including immune checkpoint inhibitors, have become the mainstay in the management of patients with lung cancer.
- However, some patients with lung cancer do not fully benefit from the current anti-tumor immunotherapy.
- A growing body of research seeks to understand immunosuppressive phenomena in terms of metabolic dysregulation within the TME.
- Immune cells infiltrating tumors can be suppressed through metabolic competition or metabolite-mediated communication by metabolically dysregulated tumor cells.
- However, despite the abundance and consistency of experimental evidence for metabolic stress of immune cells within the TME, there is a lack of data on whether this phenomenon is reproduced in tumors from patients with lung cancer.
- In particular, little is known about whether these metabolic abnormalities are observed in all lung cancers or whether they are specific to certain subtypes of lung cancer.
- Thus, this study aimed to evaluate the clinical significance of metabolic dysregulation and heterogeneity in patients with non-small cell lung cancer (NSCLC) treated with immunotherapy.

METHODS

Study population

- This study was a post hoc analysis of a prospective observational cohort conducted at the National Cancer Center Hospital (Goyang, Republic of Korea) from November 2017 to February 2021.
- The cohort study prospectively enrolled patients with stage IV lung cancer who had measurable lesions and were treated with any anti-PD-(L)1 immune checkpoint inhibitors.
- We collected tumor tissues that were obtained from patients prior to anti-PD-(L)1 treatment.

Transcriptomic analysis

- We analyzed the NanoString Metabolic Pathways Panel, which consists of 768 metabolism-related genes.
- Unsupervised clustering based on gene expression patterns was performed to define metabolic subtypes. For the functional interpretation of metabolic gene signatures, we applied both Gene Set Variation Analysis (GSVA) and Gene Set Enrichment Analysis (GSEA), to identify pathways that were differentially enriched among subtypes.
- To validate our findings, we used an independent cohort from the GSE161537 dataset comprising 64 patients diagnosed with advanced or metastatic NSCLC, who received anti-PD-(L)1 treatment as second- or third-line therapy between May 2016 and February 2018.
- Gene expression profiling was performed using an Oncology Biomarker Panel designed to measure 2,559 cancer-associated transcripts. From the full set of genes, a subset of 316 genes related to metabolic pathways was selected for focused analysis.
- All data preprocessing and statistical analyses were performed in R. Visualization of results, including heatmaps and survival plots, was performed using the R packages ggplot2 (v3.5.2) and pheatmap (RRID:SCR_016418).
- Survival time was estimated using the Kaplan–Meier method, and between-group differences in survival were assessed using the log-rank test. Two-tailed P values < 0.05 were considered statistically significant.

DISCUSSION AND CONCLUSION

Actionable Take Away Message

- This study identified five clinically relevant metabolic subtypes of NSCLC treated with immunotherapy using targeted metabolic transcriptome profiling.
- Five clinically relevant metabolic subtypes include immune signaling–dominant with minimal metabolic dysregulation (Cluster 1), amino acid metabolism–related (Cluster 2), hypoxia/autophagy–associated (Cluster 3), glycolytic/proliferative (Cluster 4), and mixed dysregulated metabolic features (Cluster 5).
- Survival outcomes differed significantly across clusters (progression-free survival, $P = 0.035$; overall survival, $P = 0.008$), with Cluster 1 showing the most favorable outcomes and Cluster 3 the worst following anti-PD-(L)1 therapy.
- Metabolic clusters were associated with clinicopathological and genomic features, including histology, smoking status, PD-L1 expression, and oncogenic driver alterations.
- Patients with high OXPHOS signature tumors showed a trend toward improved outcomes when metformin was used in combination with anti-PD-(L)1 therapy, whereas this pattern was not observed in tumors with low OXPHOS activity.
- In particular, RAS–RAF–MEK pathway gene alterations were more frequent in Clusters 3–5 than in Clusters 1–2.

Future Direction for Research

- These findings provide a framework for further investigation of metabolically defined subgroups that may inform the development of targeted therapeutic strategies.

REFERENCES & ACKNOWLEDGEMENTS

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Competing Interests

Youngjoo Lee declare no Competing Non-Financial Interests but the following Competing Financial Interests; Consulting fee: AstraZeneca, Roche, Merck, Yuhan, Bayer, and Amgen. Other Authors declare no Competing Financial or Non-Financial Interests.

Data Availability

The original NanoString discovery dataset has been deposited in the Gene Expression Omnibus (GEO) under accession number GSE309652.

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RESULTS

➤ Patient characteristics

- This study comprehensively analyzed the clinical and transcriptional data of 73 patients. The median age of the patients was 64 years (range, 47–82 years). The patients were predominantly male (79.5%) and were smokers (80.8%). Adenocarcinoma and squamous cell carcinoma accounted for 46.6% and 38.4% of the cases, respectively. Most patients (n=65, 89.0%) had previously undergone chemotherapy. Anti-PD-(L)1 treatment was administered as monotherapy to 67 (91.8%) patients.
- During the median follow-up duration of 50.2 months (95% CI, 34.9–65.5), 57 (78.1%) patients experienced disease progression and 50 (68.5%) died. The overall response rate was 34.2%. The median PFS and OS were 4.1 (95% CI: 1.5–6.7) months and 15.2 (95% CI: 10.5–19.8) months, respectively.

➤ NMF-based metabolic clustering

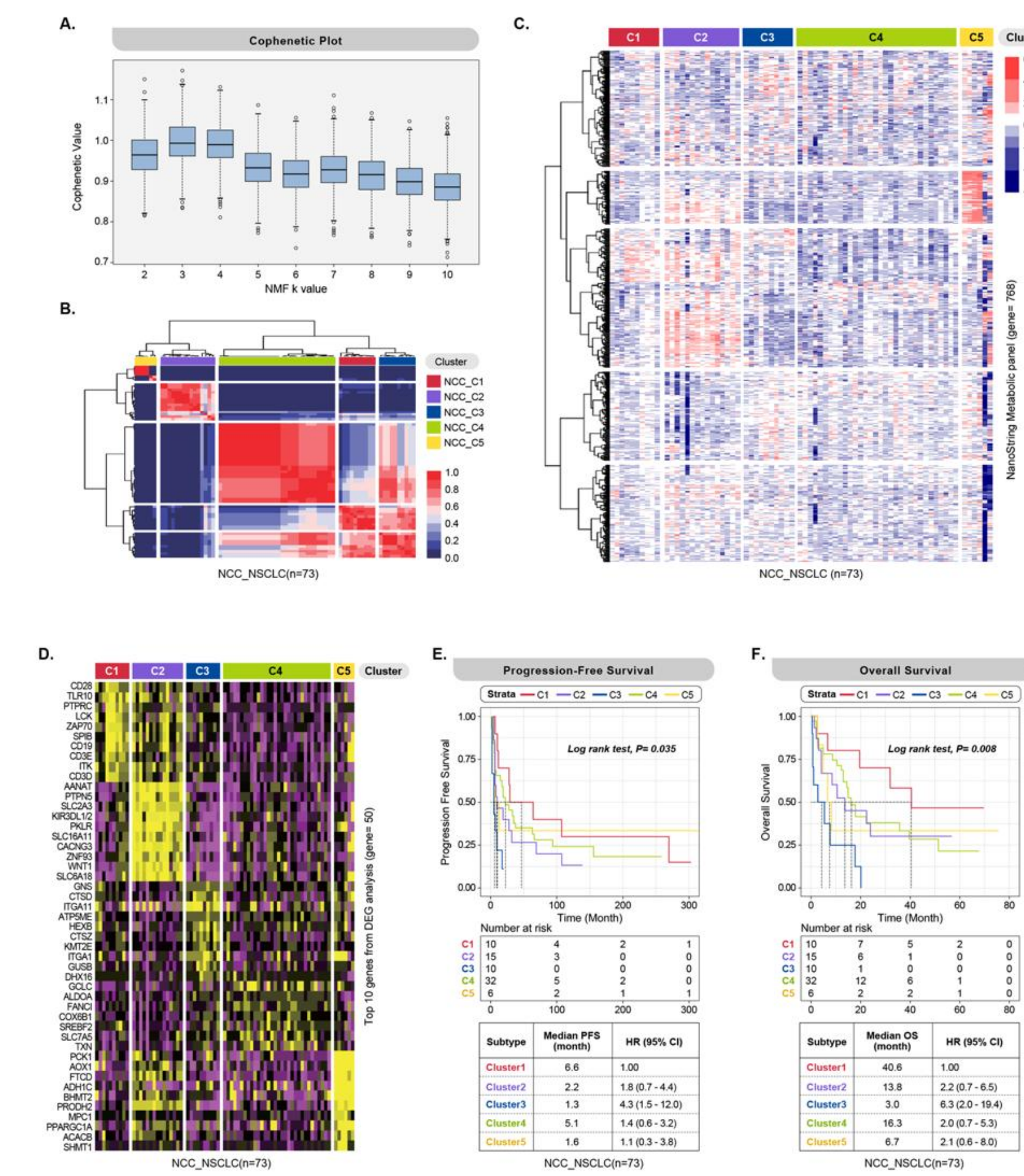


Figure 1. Identification of five metabolic pathway-based subtypes (MPS) using non-negative matrix factorization (NMF) in the NCC_NSCLC cohort (n=73).

(A) Plot of the cophenetic correlation coefficient across a range of factorization ranks (k), used to determine the optimal number of clusters for NMF-based decomposition. (B) Consensus clustering matrix heatmap for $k = 5$, showing clear and stable separation among the five metabolic clusters, each represented by distinct consensus blocks along the diagonal. (C) Heatmap of the expression levels of 768 metabolism-related genes used for NMF decomposition, grouped by the five metabolic clusters. Each cluster displays a unique transcriptomic profile reflective of distinct metabolic states. (D) Focused heatmap showing the top 10 most variably expressed genes for each cluster. (E) Kaplan–Meier curve of progression-free survival (PFS) among the five metabolic clusters. (F) Kaplan–Meier curve of overall survival (OS) among the five metabolic clusters. NCC, National Cancer Center Korea; NSCLC, non-small cell lung cancer.

➤ Metabolomic-immunologic features of subtypes

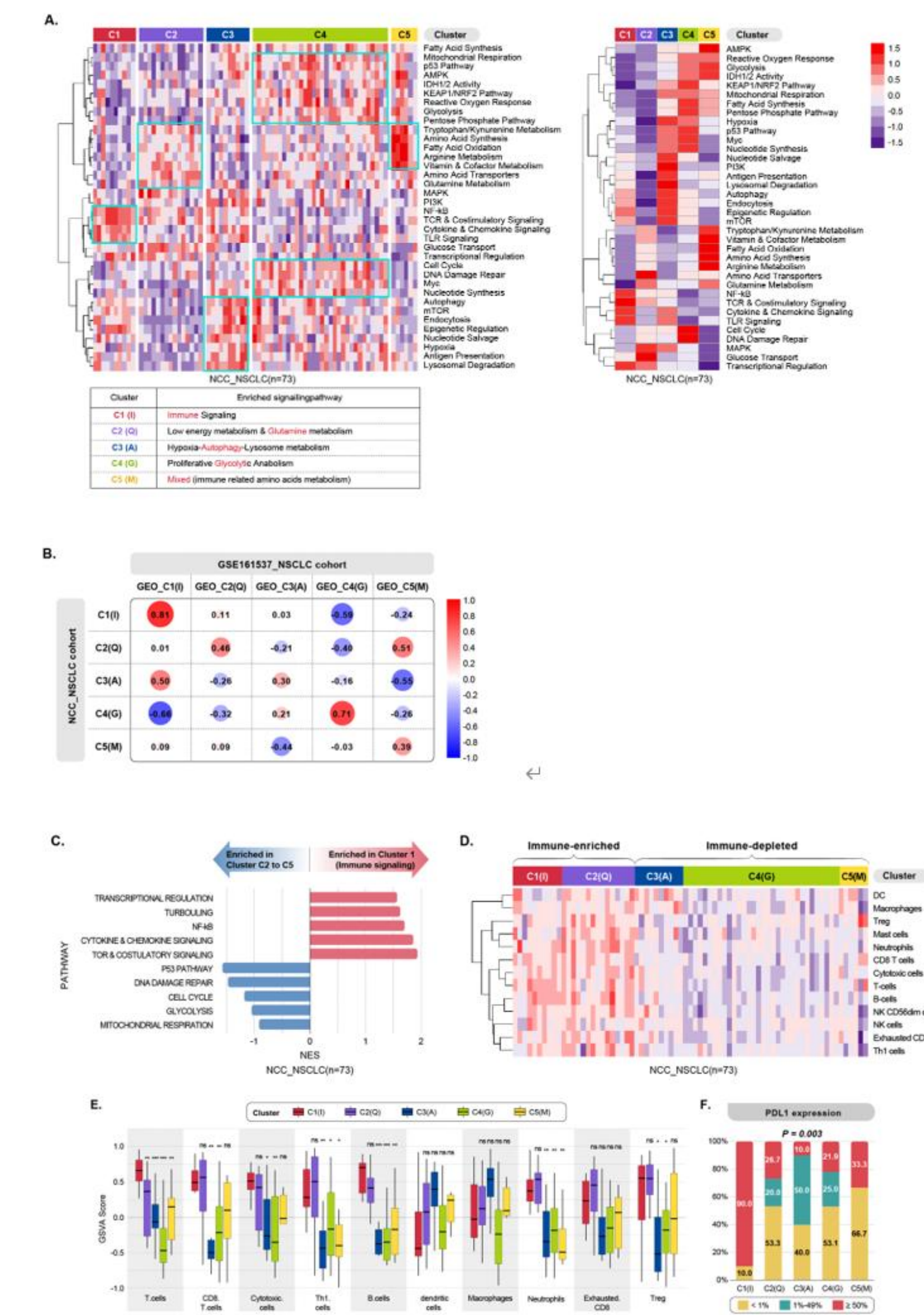


Figure 2. Characterization of five metabolic pathway-based subtypes.

(A) Heatmap showing pathway-level enrichment scores (NES) computed using GSVA using the NanoString Metabolic Pathway Panel across five metabolic clusters (Clusters 1–5) in the NCC_NSCLC cohort (n = 73). (B) Pearson correlation matrix comparing subtype-specific pathway signatures between the NCC_NSCLC and GSE161537 cohorts. (C) Gene set enrichment analysis (GSEA) comparing Cluster 2 to Cluster 5 against Cluster 1, highlighting pathway-level differences. (D) Heatmap of immune cell-type GSVA scores in the NCC_NSCLC cohort, grouped by the five metabolic clusters. The five clusters are separated into immune-enriched (Clusters 1 and 2) and immune-depleted (Clusters 3–5) groups. (E) Boxplots show GSVA enrichment scores for 10 immune-related cell-type gene sets derived from the NanoString® Metabolic Pathways Panel across five clusters. Statistical significance between Cluster 1 and each other cluster was evaluated using pairwise Wilcoxon rank-sum tests and annotated as follows: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), ns (not significant). (F) PD-L1 expression on tumor cells across the five metabolic clusters. NSCLC, non-small cell lung cancer; GSVA, gene set variation analysis.

➤ OXPHOS signature and metformin sensitivity

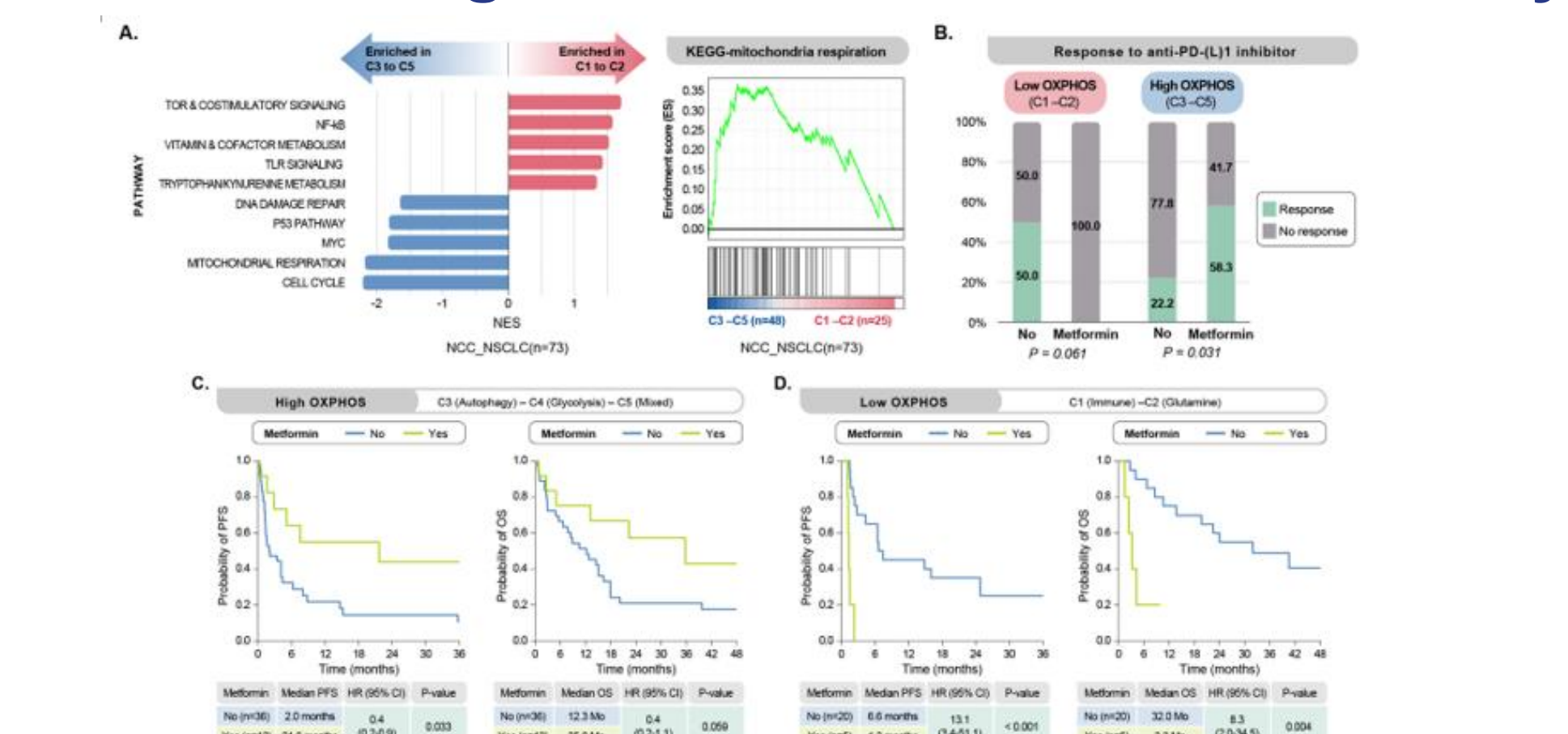


Figure 3. Clinical and transcriptomic differences based on OXPHOS signature. (A) Gene set enrichment analysis (GSEA) comparing Cluster 3 to Cluster 5 against Clusters 1 and 2, highlighting pathway-level differences. (B) Response rate to anti-PD-(L)1 inhibitor between those receiving and those not receiving metformin categorized into high (Clusters 3–5) and low (Clusters 1 and 2) OXPHOS levels. (C) Kaplan–Meier curve of progression-free survival (PFS) and overall survival (OS) between those receiving and those not receiving metformin in the high OXPHOS signature group (Clusters 3–5). (D) Kaplan–Meier curve of PFS and overall survival OS between those receiving and those not receiving metformin in the low OXPHOS signature group (Clusters 1 and 2). PD-L1, programmed cell death ligand 1; OXPHOS, oxidative phosphorylation.

➤ Clinico-pathological-genomic features of the subtypes



Table 1. Genetic alterations of OXPHOS group. No. (%)¹

Gene alterations ^{a3}	Low OXPHOS ^{a1}		High OXPHOS ^{a1}	P value ^{a2}
	C1–C2 (n=18) ^{a1}	C3–C5 (n=40) ^{a1}	C3–C5 (n=40) ^{a1}	
EGFR ^{a3}	1 (5.6) ^{a3}	6 (15.0) ^{a3}	0.417 ^{a3}	
RTK ^{a3}	1 (5.6) ^{a3}	7 (17.5) ^{a3}	0.413 ^{a3}	
PTEN-PIK3CA-AKT ^{a3}	1 (5.6) ^{a3}	3 (7.5) ^{a3}	1.000 ^{a3}	
RAS-RAF-MEK ^{a3}	0 (0.0) ^{a3}	10 (25.0) ^{a3}	0.023 ^{a3}	
RAS or PIK3CA pathway ^{a3}	1 (5.6) ^{a3}	13 (32.5) ^{a3}	0.044 ^{a3}	
Cell cycle ^{a3}	2 (11.1) ^{a3}	9 (22.5) ^{a3}	0.474 ^{a3}	
DNA repair ^{a3}	4 (22.2) ^{a3}	31 (77.5) ^{a3}	1.000 ^{a3}	
TP53 ^{a3}	8 (44.4) ^{a3}	15 (37.5) ^{a3}	0.773 ^{a3}	
Mxd ^{a3}	0 (0.0) ^{a3}	3 (7.5) ^{a3}	0.545 ^{a3}	
STK11 ^{a3}	0 (0.0) ^{a3}	2 (5.0) ^{a3}	1.000 ^{a3}	
NF1 ^{a3}	1 (5.6) ^{a3}	3 (7.5) ^{a3}	1.000 ^{a3}	
HNF1A ^{a3}	1 (5.6) ^{a3}	1 (2.5) ^{a3}	0.528 ^{a3}	
GNAS ^{a3}	1 (5.6) ^{a3}	2 (5.0) ^{a3}	1.000 ^{a3}	
CTNNB1 ^{a3}	0 (0.0) ^{a3}	2 (5.0) ^{a3}	1.000 ^{a3}	
IDH1 ^{a3}	1 (5.6) ^{a3}	1 (2.5) ^{a3}	0.528 ^{a3}	
DNMT3A ^{a3}	1 (5.6) ^{a3}	1 (2.5) ^{a3}	0.528 ^{a3}	

Abbreviations: RTK, receptor tyrosine kinase. Notes: ¹ calculated by Fisher's exact test.