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Plasma metabolic signatures uncover therapeutic response and prognosis of third-generation EGFR-TKI treatment in patients with NSCLC

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Introduction

- Advanced EGFR T790M-mutated non-small cell lung cancer (NSCLC) inevitably develops resistance to third-generation EGFR tyrosine kinase inhibitors (EGFR-TKIs) therapy.
- Metabolic reprogramming supports EGFR-TKI resistance, while thorough metabolomics clinical studies are limited.

Method

- Here, we enrolled patients from BPI-7711 Phase I and IIa clinical studies and characterize responsive metabolic features of third-generation EGFR-TKI.

Result

- Baseline plasma metabolites revealed responder (R) featured by elevated glycerophospholipid and non-responder (NR) characterized by increased unsaturated fatty acids **(Figure 1C and 1D)**.

Fig. 1. Plasma metabolic feature correlated with the response to third-generation EGFR-TKI.

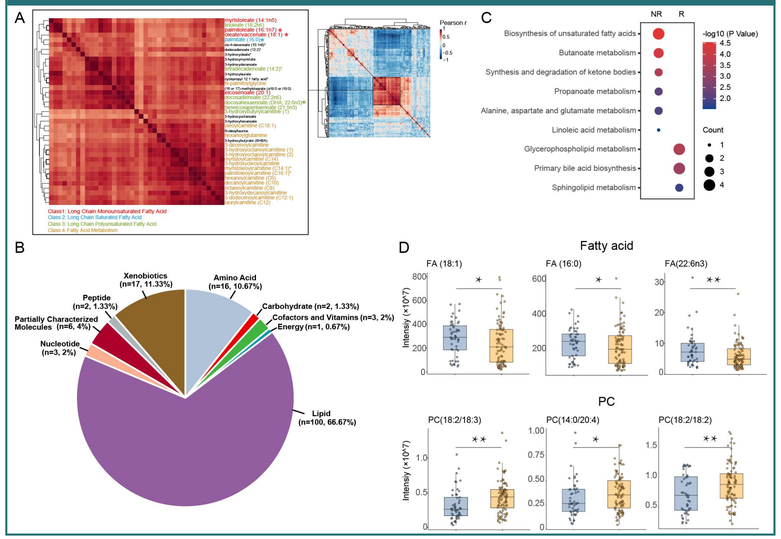

Figure 1. (A) Pairwise correlation of 951 metabolites over the 139 patients lead to a matrix of correlation coefficients. **(B)** Classes and counts of 150 differentially expressed metabolites. **(C)** Pathway enrichment analysis using metabolites significantly elevated in R or NR patients (FDR ≤0.05). R, responder; NR, non-responder; FDR, false discovery rate. **(D)** Distribution of intensity value of the top three metabolites of FA and PC. ***P < 0.001, **P < 0.01; *P < 0.05; ns, P ≥ 0.05. FA, fatty acid; PC, phosphatidylcholine

Result

- Furthermore, circulating metabolic landscape implicated two distinct metabolic subtypes, suggesting C2 with worse overall survival (OS) is characterized by higher unsaturated fatty acids **(Figure 2A, 2B and 2C)**.

Fig. 2. Plasma metabolome revealed the heterogeneity of EGFR-mutant NSCLC patients

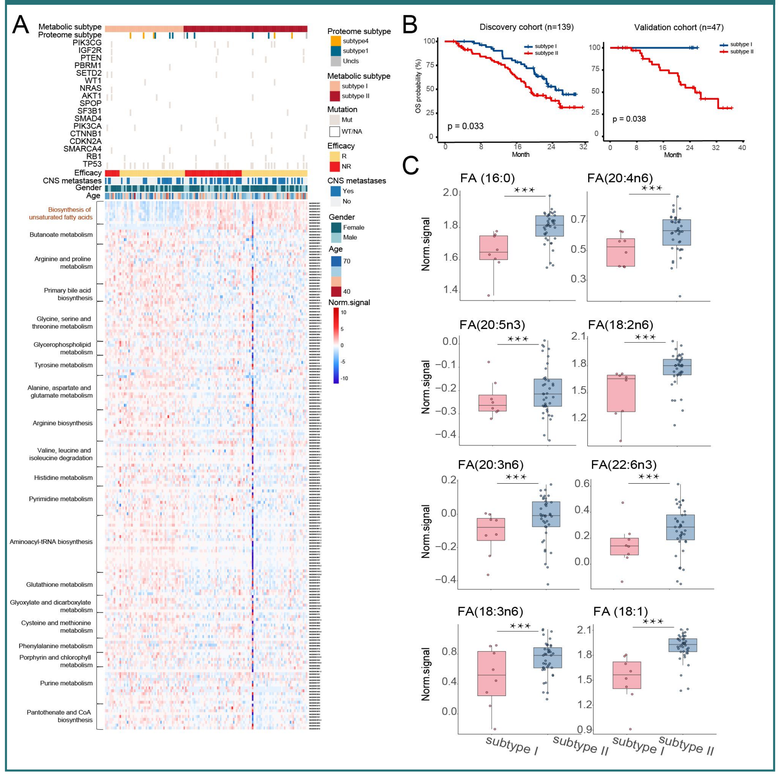

Figure 2. (A) Metabolic-based clustering of NSCLC samples (n = 139) based on 150 metabolites. Samples are indicated in columns, and metabolites are represented in rows. **(B)** Kaplan-Meier curves of OS between two subtypes in the discovery (n=139) and validation cohort (n=47). **(C)** Fatty acids were significantly elevated in subtype II compared with subtype I.

- Integrative proteomic and genomic analysis uncovered FABPs that responsible for fatty acid transport may contribute to primary and acquired resistance **(Figure 3)**.
- In vitro experiment confirmed exogenous fatty acids promote cell proliferation, reduce de nove synthesis of FAs, and increase fatty acid oxidation **(Figure 4)**.

Fig. 3. Integrative analysis of proteomics and metabolomics

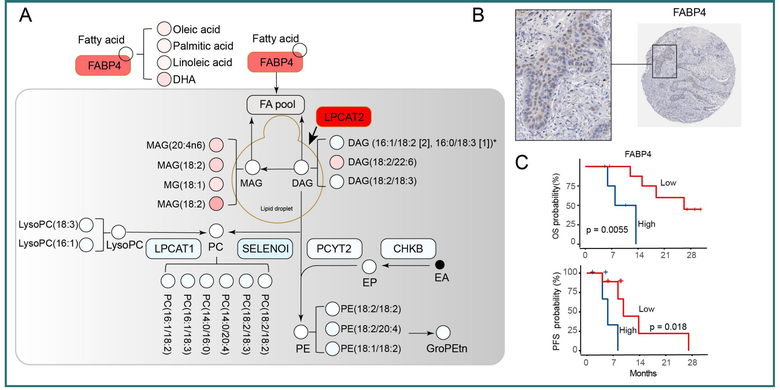

Figure 3. (A) Detailed biological signaling pathways correlated with response to third-generation EGFR-TKI. Coloring corresponds to the log2 fold change between R and NR patients. Ovals represent metabolites and rectangles represent protein levels. **(B)** Immunohistochemistry analysis of FABP4 expressing in lung cancer downloaded from the HPA database (scale bar: 50 μm). HPA, human protein atlas. **(C)** Kaplan-Meier models of PFS and OS between patient groups with high and low expression levels of FABP4.

Fig. 4. Fatty acids induce cell proliferation in a PI3K-dependent manner.

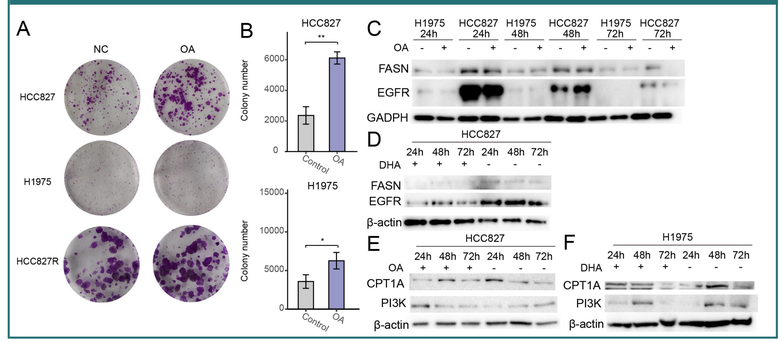

Figure 4. (A) Colony formation assays after incubation with oleic acid in HCC827, H1975 and HCC827-resistant cell lines. **(B)** Incubation with oleic acid could increased the colony number. **(C)- (F)**Time course of protein induced by FAs. HCC827 and H1975 cells were treated with 100 nmol/L OA and DHA for 24, 48, and 72 hours. β-actin was run as the internal standard for each blot to ensure equal loading.

Conclusion

- This study characterized the metabolic landscape of advanced T790M mutated NSCCL patients, providing the potential guide for personalized EGFR-TKI treatment and therapeutic target for overcoming resistance.