Glutamine Metabolism in Lung Squamous Cell Carcinoma


Altered metabolism is known to generally contribute to cancer growth, forming the conceptual basis for development of metabolic therapies as cancer treatments. However, the specific metabolic characteristics of individual cancer types in vivo are still largely unknown, limiting the translatability of metabolic therapies in the clinic. In this study we performed in vivo metabolic profiling and molecular analysis of lung squamous cell carcinoma (SCC) using both positron emission tomography and mass spectrometry. We identify a metabolic signature in this subset of lung tumors characterized by a reliance on both glucose and glutamine. Lung SCC adapt to chronic mTOR inhibition and suppression of glycolysis through the GSK3α/β signaling pathway that upregulates glutaminolysis. Phospho-GSK3α/β protein levels are predictive of response to single-therapy mTOR inhibition while combinatorial treatment with the glutaminase inhibitor CB-839 effectively overcomes therapy resistance. Lastly, we identified a conserved metabolic signature in a broad spectrum of hypermetabolic human tumors that is predictive of patient outcome and response to combined metabolic therapies targeting mTOR and glutaminase. We therefore propose a new treatment paradigm for patients with lung SCC involving the use of a metabolic signature as a biomarker to select patients who will benefit from combined therapies targeting mTOR and glutaminase.

Targeted therapies against clinically actionable oncogenic drivers in lung adenocarcinoma have significantly improved survival of cancer patients, but durable responses are limited due to the emergence of drug resistance. Resistance development is often characterized by the retention of a small subpopulation of cancer cells under drug treatment and their evolution from non-/low-proliferative residual disease to an aggressively growing resistant tumor. Most importantly, drug-tolerant persisters cells have been identified as a reservoir for a multitude of drug resistance mechanisms and thus, their characterization and the development of rational combinatorial treatment may delay or prevent resistance development and improve treatment outcome for cancer patients. Using a multitude of in vitro models such as cell culture models and patient-derived organoids, we characterized signaling and transcriptional changes in drug-tolerant persisters. We identified YAP nuclear relocalization and its increased transcriptional activity as a key marker of persisters derived from EGFR-mutant and EML4-ALK fusion-positive specimens under third-generation TKI treatment. Image analysis of cells genetically engineered via CRISPR-Cas9 to express endogenously labeled YAP-mNeonGreen validated these results. Moreover, we were able to prove the functional relevance of YAP activation in drug persistence by overexpressing active mutants of YAP that are lacking inhibitory Hippo phosphorylation sites. The latter resulted in increased nuclear levels and transcriptional activity of YAP and mediated significantly reduced cell death under high-dose drug treatment in different cell line models. Using RNA sequencing, we show a clear evolutionary path from drug-sensitive parental cells to drug-tolerant persisters and long-term derived drug-acquired resistant cells. We are currently profiling vulnerabilities of drug-tolerant EGFR-mutant and EML4-ALK fusion persisters using genetic and pharmacologic approaches. In conclusion, YAP activation is a functional marker of EGFR-mutant and EML4-ALK fusion persisters derived under high-dose drug treatment with third-generation TKIs. Targeting YAP activation either on the level of upstream signaling input, its relocalization between cytoplasm and nucleus, or its action as transcriptional coactivator may represent a promising combinatorial treatment approach to limit resistance development and improve patient survival in lung adenocarcinoma.

The GSK3 Signaling Axis Regulates Adaptive Glutamine Metabolism in Lung Squamous Cell Carcinoma

M. Momcilovic,1 J.T. Lee,1 D. Braas,1 T.G. Graeber,1 F. Parlati,2 S. Demo,3 R. Li,3 M. Gricowski,3 R. Shuman,3 J. Ibarra,3 D. Fridman,3 M. StJohn,4 N. Bernthal,4 N. Federman,4 J. Yanagawa,4 S.M. Dubinett,5 S. Sadeghi,5 H.R. Christofk,5 D.B. Shackelford4

1University of California Los Angeles, Los Angeles, CA/US, 2Calithera Biosciences, San Francisco, CA/US, 3Memorial Care Health, Long Beach, CA/US

Small-molecule tyrosine kinase inhibitors (TKIs) have become standard of care in EGFR-mutated NSCLC, but acquired resistance invariably develops due to new mutations in EGFR and activation of compensatory pathways such as cMet. JNJ-61186372 (JNJ-372) is an anti-EGFR and cMet bispecific low-fucose antibody (hulgG1) with enhanced Fc function designed to target tumors with activated EGFR and cMet signaling through a novel mechanism of action. An ongoing first-in-human study to assess the safety and efficacy of JNJ-372 in patients with advanced, treatment-refractory NSCLC revealed JNJ-372 to have clinical activity in patients with diverse EGFR-mutated NSCLC, including tumors with EGFR mutations (Exon20, T790M, C797S) resistant to TKIs and those resistant due to MET amplification. Despite observing potent antitumor activity of JNJ-372 in EGFR mutant xenograft models, only modest antiproliferative effects were observed in NSCLC cell lines in vitro. We also found that the Fc inactive version (IgG2sIgma) of the EGFR/cMet antibody was significantly impaired in its ability to inhibit tumor growth in mice compared to the Fc enhanced JNJ-372. The IgG2sIgma variant also reduced the ability of the bispecific antibody to mediate downregulation of EGFR and cMet signaling. These observations suggested that the interaction of the Fc domain of the antibody with the Fcgamma receptors on innate immune cells may play a crucial role in the mechanism of action of JNJ-372. We performed a comprehensive assessment of the Fc effector functions of JNJ-372, including effects on EGFR and cMet levels, downstream signal transduction, and role in mediating antitumor activity. Using cancer cell lines in vitro, the