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ABSTRACT

In vivo modeling combined with CRISPR/Cas9-mediated somatic genome editing has contributed to elucidating the functional importance of specific genetic alterations in human tumors. Our recent work uncovered tumor suppressor pathways that affect EGFR-driven lung tumor growth and sensitivity to tyrosine kinase inhibitors and reflect the mutational landscape and treatment outcomes in the human disease.

Tyrosine kinase inhibitors (TKIs) are the standard of care treatment for oncogenic epidermal growth factor receptor (EGFR)-driven lung adenocarcinomas. Despite the efficacy of TKIs, responses are heterogeneous and drug resistance inevitably emerges, underscoring the need to identify determinants of therapeutic sensitivity. Understanding how genotypes influence responses to drugs could help advance treatment strategies for different subsets of patients with EGFR-driven lung cancer and delay or prevent the emergence of resistance. Sequencing data of human tumors show that EGFR alterations co-occur with alterations in many putative tumor suppressor genes. Whether these alterations have biological implications and whether their relative alteration frequency reflects their functional importance remains largely unknown. Understanding the extent to which tumor suppressor gene alterations contribute to TKI sensitivity and resistance could improve treatment approaches (Figure 1). Moreover, the identification of combinations of genetic alterations that alter tumor fitness could also lead to the discovery of novel vulnerabilities of genomic subsets of EGFR mutant tumors.

Prior to our study, there were almost no in vivo studies on the function of tumor suppressor genes in oncogenic EGFR-driven lung cancer. This was at least partially due to the absence of suitable autochthonous EGFR-driven lung cancer models with which to investigate the biological consequences of gene inactivation. In our recent work, we leveraged tumor barcoding with high-throughput barcode sequencing (Tubaseq) and applied it to a novel model of EGFR mutant and Transformation related protein 53 (Trp53, best known as p53)-deficient lung adenocarcinoma to study the functional importance of tumor suppressor genes that are frequently altered in human lung tumors. Our findings revealed tumor suppressor genes that when inactivated promoted tumor growth, most notably RNA binding motif protein 10 (Rbm10), Retinoblastoma 1 (Rb1), and Adenomatous polyposis coli (Apc). Importantly, these tumor suppressor genes were also some of the most frequently altered genes in EGFR/P53 mutant human lung tumors, supporting the importance of these tumor suppressor pathways in driving EGFR mutant lung tumors.

AT-rich interaction domain 1A (Arid1a) and Cyclin-dependent kinase inhibitor 2A (Cdkn2a) inactivation appeared to increase tumor growth only later during tumor progression, suggesting that specific tumor suppressor genes can have different effects at different stages of tumor development. Inactivation of other putative tumor suppressor genes that we investigated did not promote tumor growth, indicating that the function of some tumor suppressor genes may be highly context-dependent.

Mutual exclusivity between genomic alterations in human tumors can imply redundancy of biological processes or synthetic lethality. Given the complexity of tumorigenesis, multiple factors (e.g., tumor subtype, mutational processes and load, environment) may play a role in determining genetic epistasis, suggesting that experimental approaches are particularly important for understanding why this is observed. Oncogenic EGFR alterations (mainly affecting EGFR exons 18 through 21) and oncogenic Kirsten rat sarcoma viral oncogene homologue (KRAS) missense mutations (mainly at codons 12, 13, and 61) were highly co-occurring in EGFR mutant lung tumors, suggesting that they exert a dominant-negative effect on the function of wild-type EGFR. This implies that activating EGFR alterations may serve as oncogenic drivers in EGFR mutant lung tumors, even though they do not appear to be the main drivers of tumor growth. However, further studies are needed to clarify the role of these alterations in the context of EGFR mutant lung tumors.

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Given that both these oncogenes are components of the same pathway, we anticipated that tumor suppressor genes would have similar impacts on in vivo growth of EGFR and KRAS mutant lung tumors. Although inactivation of Rbm10, Rb1, or Apc did have similar effects on EGFR and KRAS mutant tumor growth, Serine/threonine kinase 11 (Stk11, also known as Lkb1) and SET domain containing 2, histone lysine methyltransferase (Setd2) inactivation had opposite effects in the two oncogenic settings. Lkb1 and Setd2 inactivation are two of the strongest drivers of KRAS mutant tumor growth; however, their inactivation reduced EGFR mutant tumor growth. These results correlate with the relative frequency of LKB1 and SETD2 alterations in human EGFR and KRAS mutant lung tumors, suggesting the existence of a synthetic lethal relationship between Lkb1/Setd2 inactivation and oncogenic EGFR. These findings underscore the importance of quantitative modeling of genetic alterations in vivo. More broadly, the observation that inactivation of certain genes can have different effects depending on the specific oncogenic alteration present (e.g., KRAS vs. EGFR mutation) revealed surprising context specificity of the role of these genes in cancer (Figure 1).3

Osimertinib, a third-generation TKI that leads to better overall survival compared to other TKIs, has been approved as first-line therapy for patients with metastatic EGFR-driven lung cancer. Recently, osimertinib was also approved as adjuvant therapy for early-stage EGFR mutant tumors.10 Outcomes upon osimertinib treatment are variable; thus, the importance of uncovering how co-incident genomic alterations contribute to sensitivity and resistance is of great clinical relevance. We quantified the impact of inactivating 10 putative tumor suppressor genes on the response to osimertinib within our mouse model and found that Kelch-like ECH associated protein 1 (Keap1) inactivation reduced sensitivity to this therapy.2 Our in vivo data mirror clinical data suggesting that KEAP1 pathway alterations predict poor clinical responses to TKIs.3 Thus, we established a causal link between this tumor suppressor pathway and TKI sensitivity in EGFR mutant lung adenocarcinoma.

The multiplexed in vivo CRISPR/Cas9 screening approach that we used allows us to interrogate multiple genes simultaneously and assess their contributions to tumor phenotypes.3,4,5,6,8,11 Thus, it provides quantitative cause-and-effect information and avoids confounding factors that are inevitable in human tumors (e.g., high mutation burden). However, our model systems do not yet recreate the extent of genomic complexity and intratumoral heterogeneity found in human lung tumors. Future studies will also be required to uncover the molecular mechanisms by which these tumor suppressors normally constrain tumor growth and by which their inactivation sensitizes tumors to therapy. Our study represents an initial step in defining the role of tumor suppressor genes in oncogenic EGFR-driven lung tumors. We anticipate that assessing broader panels of putative tumor suppressors will further elucidate the functional genomic landscape of this disease and allow genotypes to be related to diverse cancer phenotypes, including their response to different therapies. Despite the efficacy of single agent osimertinib, quantifying the impact of rational combination therapies on defined genotypes of lung tumors will drive further gains in the treatment of specific subsets of tumors. We envision that these types of multiplexed in vivo studies will ultimately contribute to the development of tailored treatments for patients with EGFR mutant lung cancer.

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Disclosure statement

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