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Mutations in *TP53*, *PIK3CA*, *PTEN* and other genes in *EGFR* mutated lung cancers: correlation with clinical outcomes

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Abstract

Introduction—The degree and duration of response to epidermal growth factor receptor (EGFR) inhibitors in *EGFR* mutated lung cancer are heterogeneous. We hypothesized that the concurrent genomic landscape of these tumors, which is currently unknown in view of the prevailing single gene assay diagnostic paradigm in clinical practice, could play a role in clinical outcomes and/or mechanisms of resistance.

Methods—We retrospectively probed our institutional lung cancer database for tumors with *EGFR* kinase domain mutations that were also evaluated by more comprehensive molecular profiling, and evaluated tumor response to EGFR tyrosine kinase inhibitors (TKIs).

Results—Out of 171 *EGFR* mutated tumor-patient cases, 20 were sequenced using at least a limited comprehensive genomic profiling platform. 50% harbored concurrent *TP53* mutation, 10% *PIK3CA* mutation, 5% *PTEN* mutation, among others. The response rate to EGFR TKIs, the median progression-free survival (PFS) to TKIs, the percentage of *EGFR*-T790M TKI resistance and survival had higher trends in *EGFR* mutant/*TP53* wild-type cases when compared to *EGFR* mutant/*TP53* mutant tumors (all $p > 0.05$ without statistical significance); with a significantly

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CONFLICT OF INTEREST STATEMENT

Daniel B. Costa has received consulting fees and honoraria from Pfizer Inc (unrelated to the current work), consulting fees from Ariad Pharmaceuticals (unrelated to the current work), and honoraria from Boehringer Ingelheim (unrelated to the current work). Susan M. Mockus, Vanessa Spotlow, Honey Reddi, and Joan Malcolm are employees of The Jackson Laboratory that developed the JAX-Cancer Treatment Profile genomic test (related to this work).

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longer median PFS in *EGFR*-exon 19 deletion mutant/*TP53* wild-type cancers treated with 1st generation EGFR TKIs (p=0.035).

Conclusions—Concurrent mutations, specifically *TP53*, are common in *EGFR* mutated lung cancer and may alter clinical outcomes. Additional cohorts will be needed to determine if comprehensive molecular profiling adds clinically relevant information to single gene assay identification in oncogene-driven lung cancers.

Keywords

mutation; lung cancer; adenocarcinoma; EGFR; TP53; PIK3CA; PTEN; kinase inhibitor; erlotinib

INTRODUCTION

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are the evidenced-based first line palliative therapy option for advanced non-small-cell lung cancers (NSCLCs) that harbor the most common forms of *EGFR* mutations: exon 19 deletions or the exon 21 L858R [1]. The overall response rate (ORR), progression-free survival (PFS) and overall survival (OS) times favor 1st (gefitinib, erlotinib) and 2nd (afatinib) generation EGFR TKIs when compared to chemotherapy [1]. However, there is significant heterogeneity in individual patient outcomes. Some cases may respond for years while other may only respond for a few weeks, or even progress outright. The main biological mechanisms of resistance to 1st/2nd generation EGFR TKIs, either the *EGFR*-T790M mutation or oncogene bypass tracks, are also heterogeneous within different patients and even within tumors of a single patient [1–3].

It is likely that genetic, epigenetic and pharmacokinetic variations explain the marked diversity of clinical outcomes observed in clinical practice. Indeed, The Cancer Genome Atlas (TCGA) of early stage lung adenocarcinoma samples disclosed that *EGFR* mutations are concurrently present with mutations in tumor suppressor genes and oncogenes to varying degrees [4], and spatial-temporal tumor analyses have disclosed that *EGFR* mutated NSCLCs follow an evolutionary pathway with significant intratumor and/or intertumor heterogeneity [4]. Current guidelines and drug approval companion diagnostics favor limited single gene assay analysis for *EGFR* mutations in NSCLC, which has restricted our knowledge of how the most common concurrent tumor suppressor and/or oncogene mutations may impact the clinical outcomes of EGFR TKI monotherapy. Therefore, in this report we sought to probe the landscape of genomic changes that can be identified in advanced *EGFR* mutated NSCLC using commercially-available comprehensive molecular profiling platforms and correlate the co-mutation profile with response/resistance to EGFR TKIs.

METHODS

Tumor and data collection

Patient-tumor pairs followed at Beth Israel Deaconess Medical Center (BIDMC) with a diagnosis of lung cancer were registered through ongoing Institutional Review Board-approved studies [5, 6]. Pathologic data, tumor genotype, type/dose of EGFR TKI,

radiographic images and survival were assembled from retrospective chart extraction. Response Evaluation Criteria in Solid Tumors (RECIST) was utilized (version 1.0 in cases managed prior to 2010 and version 1.1 after 2010). PFS and OS were calculated from time of initiation of an EGFR TKI. Data was collected and managed using the REDCap electronic data capture held at BIDMC. The data cut-off for outcomes was May 7th 2016.

In addition, the 2014 TCGA lung adenocarcinoma mutation database [4] was reviewed and collated for *EGFR* genotypes and co-existing mutations using cBioPortal (<http://www.cbioportal.org/index.do>).

Tumor genomic analyses

EGFR, exons 18 to 21, single gene Sanger sequencing assays were performed in all diagnostic tumor samples [5]. Comprehensive genomic profiling - of selected cases beyond routine clinical care - was performed using two different commercially-available platforms: SNaPshot-NGS or JAX-Cancer Treatment Profile, as described previously [7, 8]. The gene set and exons that overlap between these next generation sequencing assays are depicted in Supplementary Table 1 and only this set was used for identification of concurrent mutation burden. We report the presence and absence of co-existing mutations in the TKI-naïve setting. Clinical and pathological characteristics of *EGFR* mutated tumors are detailed in Supplementary Table 2.

Statistical methods

Fisher's exact test was used to compare categorical variables. All p-values reported are two-sided, and tests were conducted at the 0.05 significance level. PFS and OS were analyzed using the Kaplan–Meier method and the log-rank test (Mantel-Cox) was used to compare differences in distributions. Statistical analyses and curves were performed with the GraphPad Prism 6 software (GraphPad Software, La Jolla, CA).

RESULTS

Concurrent genomic changes identified using comprehensive genomic profiling in *EGFR* mutated NSCLC

Our BIDMC database consisted of 171 *EGFR* mutated tumors identified mostly by single gene assay. Of these, 20 patient-tumor pairs were also analyzed by comprehensive genomic profiling. The majority of these tumors harbored common *EGFR* mutations in exons 19 (deletions) or L858R (15/20, 75%). The most common concurrent genomic alteration was tumor protein P53 (*TP53*), which was present in 10/20 (50%) of all *EGFR* mutated tumors (Figure 1A) and in 6/15 (40%) of tumors with *EGFR*-exon 19 deletions or L858R (Figure 1B). The second most common co-existing gene mutation was in phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*), present in 2/20 (10%) of all *EGFR* mutated tumors (Figure 1A) and in 2/15 (13.3%) of the tumors with *EGFR*-exon 19 deletions or L858R (Figure 1B). A plentitude of other gene mutations, including phosphatase and tensin homolog (*PTEN*), were identified at a frequency of <10% (Figure 1A). The frequencies of *TP53*, *PIK3CA* and *PTEN* aberrations co-occurring with *EGFR* mutations identified at BIDMC were similar to those previously reported in TCGA (Figure

1B). The spectrum of *TP53* mutations (truncating or missense mutations) that were identified in the TCGA's *EGFR* mutated lung adenocarcinoma cohort are depicted in Figure 2A. The *TP53* mutations identified at BIDMC affected similar amino-acids as the TCGA cohort (Figure 2A and Supplementary Table 2).

Clinical outcomes and mechanisms of resistance to EGFR TKIs

We further evaluated each patient's response to EGFR TKIs and subsequent mechanisms of resistance (Supplementary Table 2), with a specific focus on how *TP53* mutations impacted these parameters (Figure 2B and 2C). Out of all patients with advanced (stage IV or recurrent) NSCLCs with EGFR TKI sensitizing *EGFR* mutations that received gefitinib, erlotinib or afatinib (n=16), the ORR, median PFS and median OS were longer in patients whose tumors had wild-type (WT) *TP53* when compared to patients whose tumors were *TP53* mutated; although the differences did not reach statistical significance (Figure 2B and 2C).

When we only analyzed the most TKI responsive and common *EGFR* mutation subgroup, exon 19 deletions, the same degree of improved outcomes in tumors with concurrent *EGFR* mutation/*TP53* WT status versus *EGFR* mutation/*TP53* mutation status was noted when only studying patients that received 1st generation EGFR TKIs (Figure 2B). The most noticeable difference was in median PFS times, where *EGFR* mutated/*TP53* WT cases had a median PFS of 19 months versus a median PFS of 6.5 months for *EGFR* mutated/*TP53* mutated cases (p=0.035); as depicted in Figure 2D.

Nine patients underwent rebiopsy upon progression on EGFR TKIs. We noted that rebiopsy specimens from patients whose tumors initially lacked *TP53* mutations and with acquired resistance to 1st/2nd generations EGFR TKIs had a numerically, albeit non-statistically significant, higher rate of *EGFR*-T790M (Figure 2B).

Intratumor heterogeneity of concurrent mutations and *EGFR* mutations

To attempt to highlight the spatial-temporal relationship and nature of concurrent mutations with presumed trunk-type *EGFR* mutation, we were able to study a case in which multiple samples were available for comprehensive genomic profiling (Figure 3). This particular case of recurrent *EGFR*-L858R mutated lung adenocarcinoma was initially diagnosed as a stage I tumor with recurrence into mediastinal nodes and extra-thoracic sites two years after surgical resection. The mediastinal node recurrence was the tissue used for the initial single gene assay diagnosis of L858R and was noted to be WT for *TP53*, *PIK3CA* and *PTEN* using the robust JAX-Cancer Treatment Profile [8] comprehensive genomic profiling assay (Figure 3). The analyses of multiple sites of the original tumor, adjacent normal lung tissue and other mediastinal node recurrences disclosed heterogeneity in branch-type mutations plus the presence of germline mutations (Figure 3). Of most interest, one of the sections of the original tumor (but not all sections) harbored a *TP53* mutation that was not present in the recurrent/metastatic tumors; suggesting that some *TP53* mutations are branch-type mutations and that the current paradigm of a single biopsy analysis may be unable to highlight the complete subclonal intratumor heterogeneity of advanced cancers.

DISCUSSION

The use of comprehensive genomic profiling in clinical specimens was able to identify a significant number of concurrent mutations in *EGFR* mutated lung cancers, confirming the TCGA lung adenocarcinoma data that showed that *TP53*, *PIK3CA* and *PTEN* mutations are frequent concurrent abnormalities [4]. Although preclinical models, from our group and others, have disclosed that certain genomic changes (such as *PTEN* deletion/inactivation and/or *PIK3CA* mutation/activation/overexpression that can activate downstream pathways engaged by EGFR) can alter the pattern of response to EGFR TKIs [9], it continues to be unclear if these changes impact outcomes in the clinic due to the potential impact of intratumor heterogeneity and subclonal sampling of a single biopsy specimen [10]. Notably, examination of the spatial-temporal relationship of multiple specimens from one patient in our cohort disclosed that even *TP53* mutations may occur in only a minor subclone of one tumor area and not in other tumors, while the driver *EGFR* mutation seems to be present in all specimens (indicative of a trunk-type mutation). We also observed that even tumors with possible activating *PIK3CA*, neuroblastoma RAS viral oncogene homolog (*NRAS*) or *discoidin domain receptor tyrosine kinase 2* (*DDR2*) mutations were initially responsive to EGFR TKIs in the context of a driver *EGFR* mutation.

Despite the aforementioned concerns with intratumor heterogeneity, other groups have recently similarly reported in abstract format [11, 12] that co-occurrence of *TP53* mutation with an *EGFR* activating (TKI sensitizing) mutation in a clinical biopsy specimen may lead to inferior ORR, PFS, and OS in EGFR TKI-treated patients. Our cohort was limited by the small numbers of cases and the retrospective analysis, both of which may have limited the ability to show statistically significant differences in outcomes. Somatic mutations of *TP53* and inactivation of the TP53 protein is a pervasive event across all cancer subtypes, as TP53 is as a key tumor suppressor that has critical anti-proliferative functions [13]. The complete loss of TP53 function can accelerate the transforming potential of driver oncogenes in lung cancers [14] and hamper tumor response to cytotoxic chemotherapy [14], radiotherapy, and/or EGFR inhibitors [15]. The data on ORR, PFS, and OS presented here should be viewed as hypothesis generating. Other series will be needed to determine if inactivating *TP53* mutations (or measurements of TP53 protein levels) and other genomic changes can be established as actionable predictive and/or prognostic biomarkers in the management of advanced NSCLC harboring *EGFR* mutations.

To the best of our knowledge, ours is the first cohort to show a trend towards a decrease rate of *EGFR*-T790M as a mechanism of resistance to 1st/2nd generation EGFR TKIs in tumors with concurrent *TP53* mutations. Although a biological explanation for this observation is not apparent in preclinical models, it is possible that the absence of *TP53* mutations is a marker of a tumor with fewer genomic escape pathways that may need to mutate the EGFR signaling apparatus to bypass EGFR TKIs; hence a higher rate of *EGFR*-T790M. Conversely, more genomic complex tumors (*EGFR* and *TP53* concurrently mutated tumors) may be better equipped to activate alternate proliferative pathways that bypass EGFR as a target.

In summary, our small cohort highlights that *EGFR* mutated tumors have significant inter and intratumor heterogeneity with concurrent presence of important cancer-associated gene mutations. Some of the most common of these co-occurring changes, including *TP53* mutations, may alter clinical outcomes of EGFR TKI-treated cases. Additional cohorts will be needed to determine if routine comprehensive molecular profiling adds predictive and/or prognostic information to the contemporary single gene assay approach in oncogene-driven lung cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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HIGHLIGHTS

- *EGFR* mutations are identified concurrently with other genomic events
- The impact of concurrent mutations (*TP53*, *PIK3CA*, *PTEN* etc) on inhibitor response is unknown
- *TP53* mutations commonly co-occur with *EGFR* mutations, and may affect clinical outcomes

Figure 1. Concurrent mutations identified in *EGFR* mutated lung cancers analyzed by focused comprehensive genomic profiling panels

A Type of *EGFR* mutation and concurrent mutation profile of tumors. The graphic representation highlights indel/truncating mutation or missense mutation types. **B.** Frequency of co-occurring *EGFR*-exon 19 deletion or L858R with *TP53*, *PIK3CA* and *PTEN* in the BIDMC and TCGA cohorts.

Figure 2. *TP53* mutations and how they affect clinical outcomes or resistance to EGFR kinase inhibitors

A. Graphic representation of the spectrum of EGFR and TP53 mutations identified in the 2014 TCGA lung adenocarcinoma cohort (data obtained using cBioPortal). **B.** Response (ORR, median PFS, median OS and rebiopsy results) of EGFR TKI-treated patients whose tumors harbored or not a concurrent *TP53* mutation. **C.** Kaplan-Meier PFS curve of patients with *EGFR* mutated tumors with or without *TP53* mutations and treated with 1st/2nd generation EGFR TKIs. **D.** Kaplan-Meier PFS curve of patients with *EGFR*-exon19 deletion mutated tumors with or without *TP53* mutations treated with 1st generation EGFR TKIs. *, p-value calculated using Fisher's exact test. ^, p-values obtained using the log-rank test.

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Figure 3. Spatial-temporal heterogeneity of molecular alterations in one case of recurrent *EGFR*-L858R mutated lung adenocarcinoma prior to *EGFR* inhibitor therapy

In this case of *EGFR* mutated lung cancer, the original right upper lobectomy for a 2.0 cm invasive lung adenocarcinoma demonstrated a mixture of acinar (tumor section 1) and lepidic (tumor section 2) patterns. Both harbored the same *EGFR* mutation (L858R) as well as shared proposed germline mutations (also observed in a section of adjacent uninvolved lung parenchyma); however, significant heterogeneity was observed in branch-type mutations, including *TP53* only on tumor section 1 but not section 2. The recurrent metastatic adenocarcinoma found in two separate supraclavicular lymph nodes (lymph node 1 and 2) were morphologically similar and once again demonstrated the same driver *EGFR*-L858R mutation and shared germline mutations, but displayed significant heterogeneity in the branch-type mutations.