Analysis of Tumor Genomic Pathway Alterations Using Broad-Panel Next-Generation Sequencing in Surgically Resected Lung Adenocarcinoma

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Abstract

Purpose: The majority of broad-panel tumor genomic profiling has used a gene-centric approach, although much of that data is unused in clinical decision making. We hypothesized that a pathway-centric approach using next-generation sequencing (NGS), combined with conventional clinicopathologic features, may better predict disease-free survival (DFS) in early stage lung adenocarcinoma.

Experimental Design: Utilizing our prospectively maintained database, we analyzed 492 patients with primary, untreated, completely surgically resected lung adenocarcinoma. Ten canonical pathways were analyzed using broad-panel NGS. The correlations of DFS and number (and type) of pathway (NPA) were analyzed using the Kaplan–Meier method and log-rank test. Associations between altered pathways and clinicopathologic variables, as well as identification of actionable therapeutic strategies were explored.

Results: Median NPA for the cohort was two (range, 0–5). Smoking status, solid morphologic appearance on preoperative CT, maximal standardized uptake value, pathologic tumor size, aggressive histologic subtype, lymphovascular invasion, visceral pleural invasion, and positive lymph nodes were significantly associated with NPA ($P < 0.05$). Of 543 actionable genetic alterations identified, 455 (84%) were within the RTK/RAS pathway. A total of 86 tumors had actionable therapeutic genomic alterations in >1 pathway. On multivariable analysis, higher NPA was significantly associated with worse DFS (HR, 1.31; $P = 0.014$).

Conclusions: NPA and specific pathway alterations are associated with clinicopathologic features in patients with surgically resected lung adenocarcinoma. Cell cycle, Hippo, TGFβ, and p53 pathway alterations are associated with poor DFS. Finally, NPA is an independent risk factor for poor DFS in our cohort.

See related commentary by Blakely, p. 7269

Introduction

Broad-panel genomic sequencing is increasingly used to select targeted therapies and precision medicine strategies for patients with lung cancer (1). However, a recent study found that <5% of patients who underwent broad-panel genomic sequencing benefited from it, compared with patients who received only EGFR and/or ALK testing (2). This raises the possibility that much of the information included in genomic sequencing panels is currently not well-utilized. Heretofore, the majority of tumor genomic analyses have focused on gene-centric approaches that detect driver alterations and identify functionally irrelevant passenger events, particularly in solid tumors with higher mutational burdens (3). Cancer cell biology and its resulting clinical phenotypes are driven by different pathways, many of which have upstream oncogenic alterations that lead to similar downstream alterations and phenotypes within the same pathway (3, 4). Therefore, a pathway-centric approach has been proposed to better identify functional alterations in selected oncogenic pathways, to explore cooccurrence and mutual exclusivity between pathways, and to identify relevant pathway alteration(s) that may be therapeutically exploited. We recently examined The Cancer Genome Atlas (TCGA) and comprehensively identified 10 canonical pathways covering 89% of 9,125 different solid tumor types, which now provides a curated and standardized pipeline to perform pathway-centric next-generation sequencing (NGS) studies (5).

Lung adenocarcinoma is a genomically well-annotated malignancy with a high background mutational burden. The majority of lung adenocarcinoma genomic analyses have focused on

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AACR 7475

Translational Relevance

Broad-panel tumor genomic profiling typically uses a gene-centric approach for analysis and to guide clinical decision making, although most of the genomic data remain unused. We hypothesized that a pathway-centric approach using next-generation sequencing (NGS) may offer additional information to identify patients at high risk of recurrence following resection of lung adenocarcinoma. We interrogated 492 lung adenocarcinoma specimens and identified number of pathway alterations (NPA) to be independently associated with disease-free survival. Moreover, NPA and specific pathway perturbations were associated with clinicopathologic features of tumors at high risk of recurrence. Finally, we identified specific pathway alteration cooccurrences that implicate possible functional synergies for targeting both pathways in patients at high risk of recurrence. Collectively, these observations highlight the prognostic importance and potential discovery of therapeutic vulnerabilities when a tumor genomic pathway-centric analysis is utilized using broad-panel NGS.

metastatic disease (6), with the 2014 TCGA analysis the only study to examine genomic changes in earlier stage lung adenocarcinoma (7). While surgical resection is the preferred treatment for early stage lung adenocarcinoma, there remains a relatively high recurrence rate, even in pathologic node-negative disease (8–12). Therefore, a significant knowledge gap exists in identifying patients at risk for recurrence following complete surgical resection of lung adenocarcinoma. To address this, we performed a comprehensive analysis to investigate associations between tumor genomic pathway alterations and selected clinicopathologic features and disease-free survival (DFS) in patients with lung adenocarcinoma who have undergone complete resection.

Materials and Methods

Patient cohort

This study was approved by the Institutional Review Board at Memorial Sloan Kettering. Using our prospectively maintained database, we identified patients who underwent complete surgical resection (R0) for lung adenocarcinoma and had targeted NGS (MSK-IMPACT) performed on their primary tumor between February 2008 and January 2018. Exclusion criteria included induction therapy, microscopic or macroscopic residual disease (R1/R2 resection), low-quality NGS, stage IV disease, and mixed tumor type (Supplementary Fig. S1).

Clinical characteristics, preoperative CT, PET images, and pathologic reports [8th edition American Joint Committee on Cancer (AJCC) Cancer Staging Manual] were reviewed. Follow-up was performed in accordance with NCCN guidelines (13). Recurrences were distinguished from metachronous tumors using Martini and Melamed criteria, with confirmation from pathologic and genomic relatedness when available (14).

MSK-IMPACT sequencing

Sequencing for MSK-IMPACT was performed as described previously (15). Patient clinicopathologic data were matched with genomic data and visualized using the cBioPortal for Cancer

Genomics (16, 17). Tumor DNA and corresponding patient-matched blood DNA were extracted. All exons and selected introns were sequenced using the MSK-IMPACT panel to identify somatic alterations, copy number alterations, and mutations. Median sequencing coverage was 764X (range, 164–1,424). Selected panel sizes have been used over time (341-, 410-, and 468-gene panels for 14, 228, and 250 patients, respectively). Tumor mutational burden (TMB) was defined as the total number of nonsynonymous single-nucleotide or insertion/deletion mutations divided by the number of Mbs in the coding region captured by each panel (0.98, 1.06, and 1.22 Mb in the 341-, 410-, and 468-gene panels, respectively; ref. 18). We have previously shown that TMB calculations using this NGS panel are strongly associated with the TMB assessed by whole-exome sequencing (18). The fraction of genome altered (FGA) was defined as the fraction of $\log_2$ copy number variation (gain or loss) >0.2 divided by the size of the genome whose copy number was profiled. FGA was corrected for tumor purity, ploidy, and clonal heterogeneity using the FACET method (19).

Pathway alteration and therapeutic actionability identification

We evaluated 10 canonical signaling pathways using the templates provided in the signaling pathways manuscript from the TCGA PanCancer Atlas project (5). The pathways analyzed were (i) cell cycle, (ii) Hippo, (iii) Myc, (iv) Notch, (v) oxidative stress response/Nrf2, (vi) PI3K, (vii) receptor tyrosine kinase (RTK)/RAS/MAPK, (viii) TGFβ, (ix) p53, and (x) β-catenin/Wnt. In total, 109 genes were identified at the intersection of the *a priori* pathway templates (5) and the MSK-IMPACT panel (Supplementary Table S1). A tumor was considered "altered" in the specific pathway when ≥1 gene relative to control in the corresponding pathway template was altered. The status of specific pathways was determined to be either altered or wild-type for each patient. Number of pathway alterations (NPA) was calculated as the total number of altered pathways out of the 10 identified pathways for each patient.

Therapeutic actionability information was annotated using OncoKB on June 5, 2018 (20). Each potentially actionable genomic alteration was stratified into 1 of 6 levels. Detailed methods used for stratification of therapeutic actionable genetic events are included in the Supplementary Materials and Methods. *CDK4* and *MDM2* genomic data from the TCGA PanCancer Atlas lung adenocarcinoma cohort (6) were accessed using cBioPortal.

Statistical analysis

Associations between clinicopathologic and genomic characteristics, specific pathway alterations, and NPA were analyzed using Fisher exact test or Cochran–Armitage test for categorical variables and Wilcoxon rank-sum test or Spearman rank correlation test for continuous variables. When comparisons were repeatedly performed across 10 pathways, *P* values were adjusted using the FDR method when needed.

The primary outcome of interest was DFS, defined as the duration between surgery and recurrence or death without recurrence. Patients were censored at the last follow-up. Median follow-up was calculated on the basis of the reverse Kaplan–Meier approach (21). DFS was estimated using the Kaplan–Meier approach and compared between clinicopathologic characteristics and pathway alterations using log-rank tests, stratified by pathologic stage where appropriate. For the DFS analysis, NPA was considered a linear factor, as there was inadequate evidence to

reject the linearity assumption (Wald F-Statistic $\chi^2 = 0.09$; $P = 0.8$) using restricted cubic splines.

The primary objective was to quantify the prognostic value of NPA. The relationships between NPA and clinically relevant factors were quantified using Cox proportional hazards models, stratified by pathologic stage [except in the cases of stage, tumor size, lymph node status, and visceral pleural invasion (VPI)]. A multivariable model was constructed starting with all factors with $P < 0.1$ in the univariable analyses. Multiple imputations were conducted to address missing data (details in Supplementary Data). To avoid loss of information by using the simple summary of NPA, we compared an NPA-only model with a model that included all 10 pathways as individual variables in a multivariable penalized Cox model. Performance of each model was quantified as discrimination (C-index) or calibration (details in Supplementary Data).

Pathway or gene mutual exclusivity or cooccurrence was analyzed using Fisher exact test, and P values were adjusted (FDR method). All analyses were two-sided, and $P < 0.05$ was considered statistically significant. Statistical analyses were conducted using Stata 13.1 (Stata) and R 3.5.1.

Results

In total, 492 patients met the inclusion criteria, of whom 356 (72%) had stage I disease. Median follow-up was 19 months [95% confidence interval (CI), 18.4–20.1]. Alterations in common lung adenocarcinoma driver genes included 40% *KRAS*, 26% *EGFR*, 5% *BRAF*, 1.6% *ALK*, 0.8% *ROS1*, and 0.6% *MET* (Fig. 1A). Alterations in these driver genes were found to be evenly distributed across all NPA groups. The alteration frequencies of the 109 genes utilized for pathway analysis are included in Supplementary Table S2. Median NPA was 2 (range, 0–5; Fig. 1A; Table 1). The most and least frequently altered pathways were RTK/RAS [$n = 415$ patients (84%)] and TGFβ [$n = 13$ patients (3%)]. Of the 164 patients with only one pathway alteration, 141 (86%) had the RTK/RAS pathway altered. Comparatively, 90% of patients (160/178) with NPA = 2, 91% (86/94) with NPA = 3, 85% (23/27) with NPA = 4, and 83% (5/6) with NPA = 5 had the RTK/RAS pathway altered. The RTK/RAS pathway was divided into two pathways as a function of NPA to better visualize the alteration frequencies of individual genes in the RTK and RAS/RAF pathways (Supplementary Fig. S2). Median normalized TMB was 4.7 (range, 0–164.2), and median FGA was 4.3% (range, 0%–49.8%).

Pathway alterations and genomic features

TMB and FGA are essential genomic features reported to be associated with survival and recurrence in patients with cancer (22–25). We investigated the correlation of TMB and FGA with pathway alterations and found, as NPA increased, TMB ($\rho = 0.50$; $P < 0.001$; Fig. 1B) and FGA ($\rho = 0.31$; $P < 0.001$; Fig. 1C) increased correspondingly. However, the correlation between TMB and FGA was significant, but the magnitude of correlation ($\rho = 0.12$; $P = 0.006$) is considered negligible on the basis of the interpretation (26) by Hinkle and colleagues (Fig. 1D).

We next examined the association between TMB, FGA, and individual pathway alterations (Supplementary Table S3). Tumors with cell-cycle, Hippo, Myc, Notch, Nrf2, PI3K, p53, or Wnt pathway alterations had significantly higher TMB than

tumors without these pathway alterations (Fig. 1E). Similarly, tumors with cell-cycle and p53 pathway alterations had significantly higher FGA than tumors without these pathway alterations (Fig. 1F).

Pathway alterations and clinicopathologic features

We then investigated associations between NPA and selected poor-risk clinicopathologic factors (Fig. 2A–H). As NPA increased, the rate of ever-smokers also significantly increased (Cochran–Armitage test, $P < 0.001$). Similar analyses showed increasing proportion of solid tumor morphologic appearance on preoperative CT, PET tumor maximal standardized uptake value (SUV$_{max}$; $\rho = 0.28$; $P < 0.001$), pathologic tumor size ($\rho = 0.20$; $P < 0.001$), aggressive histologic subtype (micropapillary or solid; Cochran–Armitage test, $P < 0.001$), lymphovascular invasion (LVI; Cochran–Armitage test, $P < 0.001$), VPI (Cochran–Armitage test, $P = 0.04$), and lymph node status (Cochran–Armitage test, $P = 0.001$) were all associated with increasing NPA.

Finally, we investigated the association between clinicopathologic features and individual pathway alterations (Supplementary Table S3, Supplementary Fig. S3). Nrf2 and PI3K pathway alterations were associated with smoking status. Cell-cycle, Hippo, Nrf2, PI3K, p53, and Wnt pathway alterations were associated with solid tumor morphologic appearance on CT scan. Cell-cycle, p53, and Wnt pathway alterations were associated with higher tumor SUV$_{max}$. Cell-cycle, p53, and Wnt pathways were associated with pathologic tumor size. P53 pathway alteration was associated with aggressive histologic subtype and LVI.

Pathway alterations and DFS

A DFS event occurred in 79 individuals, with 70 (89%) experiencing recurrence of their disease. The recurrence rate was 33% for patients with ≥4 NPA and 9% for those with 0 NPA (Fig. 2I). The median DFS for patients with ≥4 NPA was 25.6 months (95% CI, 9.7–41.6); patients with 0 NPA had a median DFS of 89.4 months (95% CI, 10.7–168.1; Fig. 2J). We next examined pathologic stage–specific DFS on the basis of NPA and observed that increasing NPA was associated with worse DFS in stages I, II, and III lung adenocarcinoma (Supplementary Fig. S4). Altered pathways were associated with worse 2-year DFS (95% CI) versus wild-type pathways for cell cycle [70.5% (59.0%–84.2%) vs. 82.4% (77.6%–87.5%); log rank $P = 0.03$], Hippo [38.9% (14.8%–100.0%) vs. 81.6% (77.1%–86.4%); log rank $P = 0.046$], TGFβ [21.2% (4.0%–100.0%) vs. 82.2% (77.8%–86.8%); log rank $P < 0.001$], and p53 [70.9% (63.3%–79.4%) vs. 87.0% (81.8%–92.7%); log rank $P < 0.001$; Fig. 3]. On univariable analysis, when stratified by stage, increase in NPA was associated with worse DFS [HR, 1.38 (95% CI, 1.12–1.69); $P = 0.002$; Table 2]. Other clinicopathologic factors, including solid tumor morphologic appearance, tumor SUV$_{max}$, pathologic tumor size, pathologic stage, LVI, predominant histologic subtype, VPI, lymph node status, and FGA, were significantly associated with DFS in univariable models (Table 2). However, subsequent multivariable analysis revealed only NPA [HR, 1.31 (95% CI, 1.06–1.62); $P = 0.01$], solid tumor morphologic appearance, tumor SUV$_{max}$, and pathologic stage were independently associated with DFS (Table 2).

We next compared the simple pathway analysis model (simple-PA model; includes only NPA) with the complex pathway analysis
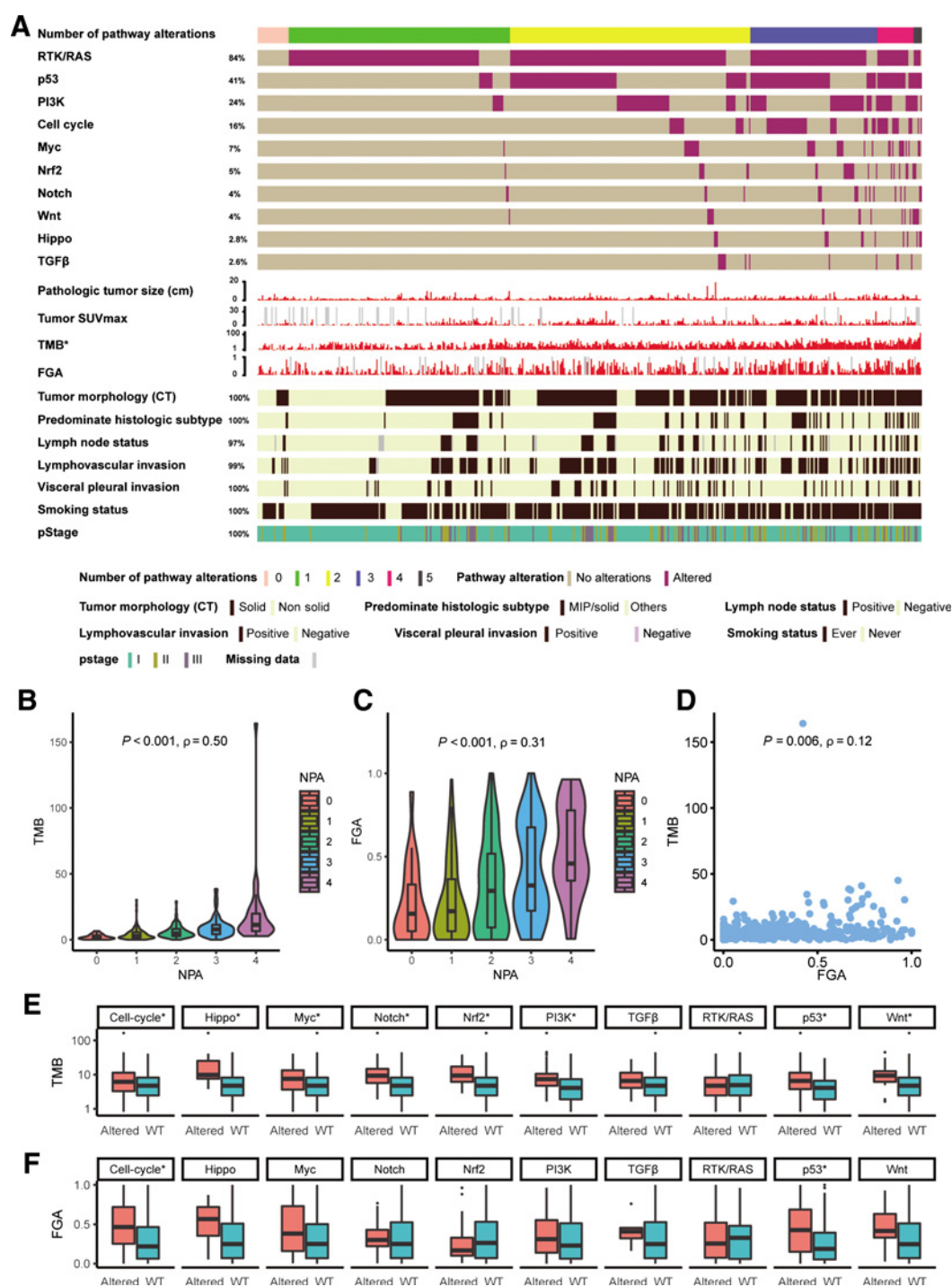
Figure 1.
Association between pathway alterations and genomic features. **A,** Oncoprint of all patients. **B,** Violin plot of TMB versus NPAs. **C,** Violin plot of FGA versus NPA. **D,** Scatterplot of TMB versus FGA. Bar plots of TMB (**E**) and FGA (**F**) versus pathway alteration status [altered and wild-type (WT)]. SE displayed as error bar. *, logarithmic scale.

model (complex-PA model; includes the 10 pathways; Supplementary Table S4) in terms of performance by quantifying their discrimination and calibration. The cross-validated C-indexes were 0.658 (95% CI, 0.576–0.732) and 0.657 (95% CI, 0.530–0.750) for the simple-PA and complex-PA models, respectively. Bias-corrected calibration curves confirmed the simple-PA model was well-calibrated, whereas the complex-PA model tended to underestimate DFS (Supplementary Fig. S4).

Table 1. Patient characteristics ($N = 492$)

Characteristic	No. (%)
Age at surgery, years, median (range)	69 (34–87)
Sex	
Male	326 (66)
Female	166 (34)
Smoking status	
Ever	380 (77)
Never	112 (23)
Pack-years[a]	20 (0–120)
Tumor morphologic appearance, CT criteria	
Pure ground glass opacity	72 (15)
Mixed ground glass opacity/subsolid	90 (18)
Solid	330 (67)
Tumor maximal standardized uptake value, median (range)	3.4 (0–31)
Procedure type	
Lobectomy	340 (69)
Segmentectomy	47 (9)
Wedge	102 (21)
Pneumonectomy	3 (1)
Pathologic tumor size, cm, median (range)	1.8 (0.08–19.5)
Predominant histologic subtype	
Lepidic, AIS, MIA	73 (15)
Acinar	263 (53)
Papillary	37 (8)
Micropapillary	32 (7)
Solid	60 (12)
Other	27 (5)
Lymphovascular invasion	
Positive	302 (62[a])
Negative	185 (38[a])
Visceral pleural invasion	
Positive	77 (16[a])
Negative	414 (84[a])
Lymph node status	
Positive	83 (17[a])
Negative	394 (83[a])
Pathologic stage	
I	356 (72)
II	80 (16)
III	56 (11)
Pathway alteration	
Cell cycle	77 (16)
Hippo	14 (3)
Myc	36 (7)
Notch	19 (4)
Nrf2	27 (5)
PI3K	119 (24)
TGFβ	13 (3)
RTK/RAS	415 (84)
p53	201 (41)
Wnt	19 (4)
Number of pathway alterations	
0	23 (5)
1	164 (33)
2	178 (36)
3	94 (19)
4	27 (6)
5	6 (1)
TMB, median (range) (IQR)	4.7 (0–164.2) (2.5–8.2)
FGA, median (range) (IQR)	0.25 (0–1) (0.1–0.5)

Abbreviations: AIS, adenocarcinoma *in situ*; IQR, interquartile range; MIA, minimally invasive adenocarcinoma.
[a]Denominator excluding patients with missing data.

Mutual exclusivity, cooccurrence, and therapeutic actionabilities

The mutual exclusivity of a pathway or gene may reflect functional redundancy or synthetic lethality (27, 28), whereas cooccurrence(s) may reflect functional synergies important in resistance to targeted therapies (29). Of the 10 pathways investigated, two pairs, p53–cell cycle (OR, 5.78; $P < 0.001$) and PI3K-Nrf2 (OR, 7.17; $P < 0.001$), cooccurred with statistical significance (Fig. 4A). We found no mutual exclusivity among pathways in our cohort. Within the p53–cell cycle and PI3K-Nrf2 pathways, alterations in three pairs of genes, *CDK4-MDM2* ($\log_2$ OR > 3; $P < 0.001$), *RB1-TP53* ($\log_2$ OR = 2.81; $P = 0.03$), and *STK11-KEAP1* ($\log_2$ OR > 3; $P < 0.001$), cooccurred with statistical significance (Fig. 4B).

Cooccurrences of *CDK4* (cell-cycle pathway) and *MDM2* (p53 pathway) amplification were significant ($n = 26$, cell cycle; $n = 30$, p53; $n = 16$, both; Fig. 4C). This pair of genes also has corresponding level 2B (palbociclib and abemaciclib for *CDK* amplification) and level 4 (DS-3032b and RG7112 for MDM2 amplification) actionable drugs. This observation suggests patients with tumors with cooccurent *CDK4* and *MDM2* amplifications may benefit from combined therapies that target both pathways. Supporting our observation, analysis of the TCGA lung adenocarcinoma cohort (5) reveals these genes have a significant cooccurrence ($\log_2$ OR > 3; $P < 0.001$; Fig. 4C).

Finally, using OncoKB, we investigated the therapeutic actionability of selected genomic alterations. We identified 543 actionable genomic alteration events across 437 tumors (Fig. 4D). A list of tumors with targetable alterations at the pathway and gene level with associated therapies and levels of evidence as curated using OncoKB can be found in Supplementary Table S5. Of these 543 actionable alterations, 455 (84%) were within the RTK/RAS pathway, including all level 1 and level 2A actionable alterations. Interestingly, tumors with RTK/RAS pathway alterations had the lowest mean NPA ($n = 2$) and the highest proportion of tumors with 1 NPA (Fig. 4D and E). Of the 437 tumors with actionable alterations, 351 had actionable therapeutic alterations in only one pathway; the other 86 had actionable therapeutic alterations in >1 pathway (Fig. 4F).

Discussion

To our knowledge, this is the first study to comprehensively investigate the association between selected pathway alterations and detailed clinicopathologic features and DFS in patients with completely resected lung adenocarcinoma using broad-panel NGS. Ninety-five percent of our cohort had at least one pathway alteration, and our analysis used 109 genes, of which 57 were altered in <2% of patients. This "long tail" distribution (30–32) of altered genes may complicate the identification of the functionally relevant driver genes from the passenger genes. However, as we show, aggregating these genes into biologically relevant molecular pathways permits the identification of associations with relevant clinicopathologic features and DFS.

It is unknown which pathway alteration(s) initiate the progressive evolution of normal tissue along the continuum toward a neoplastic state. Our results revealed that, among patients with 1 NPA, 86% had an alteration in the RTK/RAS pathway. Of the 10 oncogenic pathways studied, the RTK/RAS pathway comprised the greatest number of clinically relevant genes ($n = 38$), including three common driver genes in lung adenocarcinoma (*KRAS*, *EGFR*, and *BRAF*). Activation of the RTK/RAS pathway leads to cell proliferation and growth (33), which are fundamental for tumor cell development (34, 35). This may explain the high frequency of
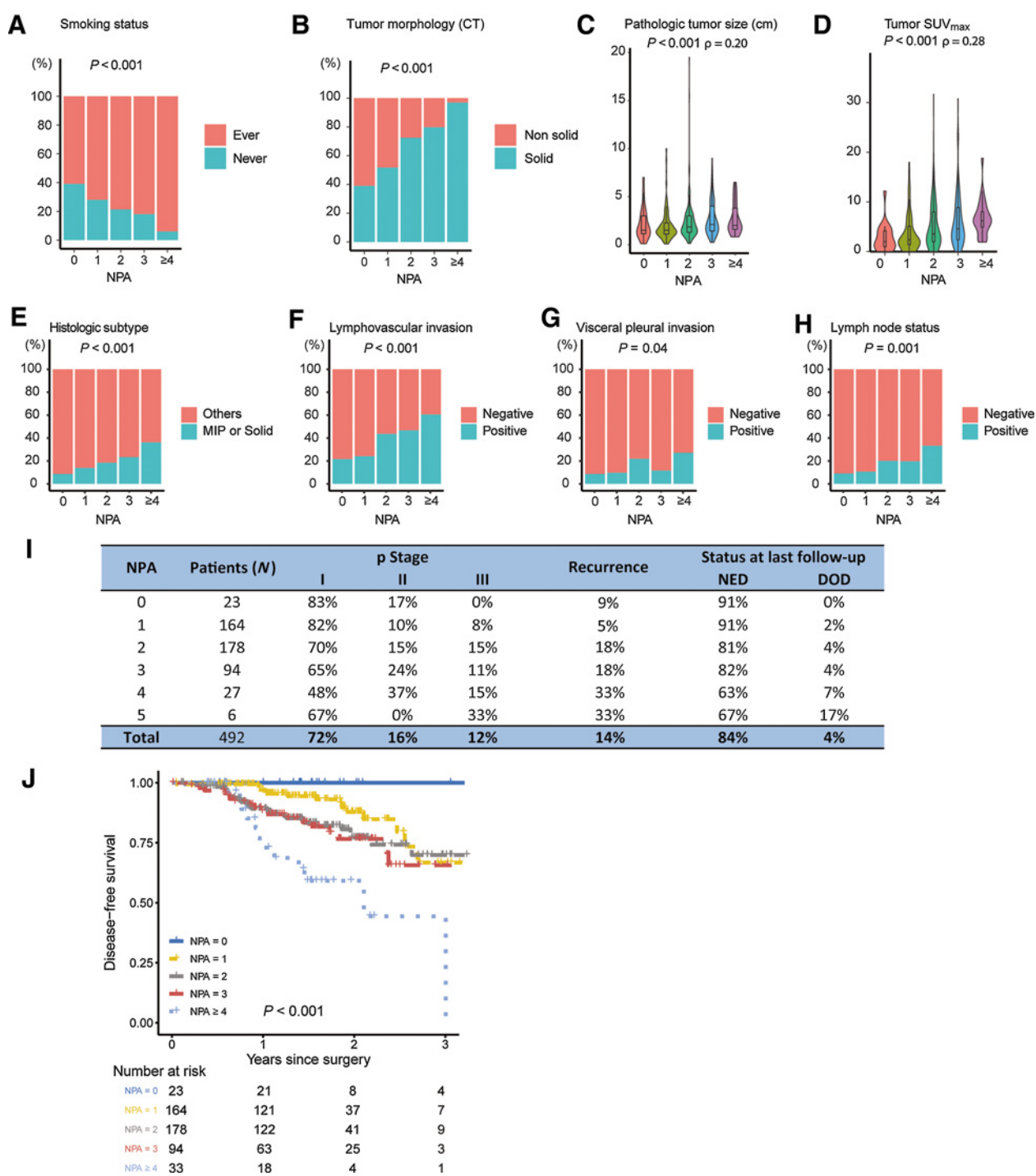
Figure 2.
NPAs and clinicopathologic features and DFS. Association between NPA and smoking status (**A**), morphologic appearance on CT (**B**), pathologic tumor size (**C**), tumor maximal standardized uptake value (SUV$_{max}$; **D**), predominate histologic subtype (**E**), lymphovascular invasion (**F**), visceral pleural invasion (**G**), and lymph node status (**H**). **I,** Staging and outcome distribution among different NPA groups. DOD, dead of disease; NED, no evidence of disease. **J,** Association between DFS and NPA.

RTK/RAS pathway alteration in our study cohort. Similar findings were reported in the lung adenocarcinoma TCGA cohort (5). Furthermore, the proportion of patients with alterations in the RTK/RAS pathway remained stable across all NPA groups. This suggests RTK/RAS pathway activation may be an early event in lung adenocarcinoma. However, this finding warrants further validation from dynamic tumor genomic profiling and multisite tumor sequencing.
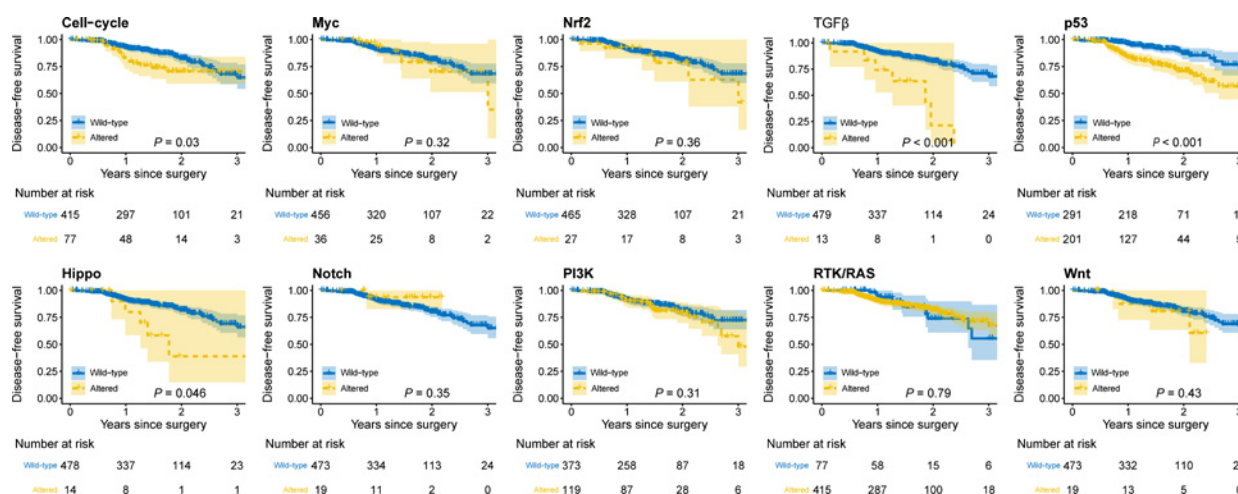
Figure 3.
Associations of individual pathway alterations and DFS.

Increases in NPA were significantly associated with increases in TMB ($\rho = 0.50$; $P < 0.001$) and FGA ($\rho = 0.31$; $P < 0.001$). This suggests that, as more mitogenic signaling pathway alterations occur, there may also be more gene replication errors (such as gene mutations or copy number alterations). However, although significant, the magnitude of correlation (Spearman correlation coefficient = 0.12) between TMB and FGA is considered negligible on the basis of the interpretation (26) by Hinkle and colleagues. Neither was shown to be an independent predictor of DFS on multivariable analysis. In a pathway-centric analysis, some pathways, such as cell cycle, are more copy number driven, whereas other pathways, such as TGFβ, are more mutation driven (5). This suggests TMB and FGA can each play a unique role in assessing therapeutic response and clinical outcomes in patients with lung adenocarcinoma. For example, higher TMB is associated with potentially better efficacy of immunotherapy (18), whereas higher FGA may be associated with worse outcomes following immunotherapy (36). Finally, high TMB has been associated with

conflicting prognoses in lung cancer (23, 25), whereas increasing FGA has been associated with worse outcomes in prostate, breast, and colorectal cancers (24, 37, 38).

Increases in NPA were significantly associated with tumor SUV$_{max}$, solid morphologic appearance on preoperative CT, pathologic tumor size, aggressive histologic subtype, LVI, VPI, and pathologic lymph node status. All these clinicopathologic features have been previously associated with poor outcomes following complete resection of lung adenocarcinoma (11, 39, 40). Finally, we found that increasing NPA was independently associated with worse DFS.

Analysis of mutual exclusivity and cooccurrence revealed interplay within and across pathways. When these findings were combined with therapeutic actionability from a pathway-centric perspective, we found that 84% of actionable alterations were located within the RTK/RAS pathway, and tumors with RTK/RAS pathway alterations had the lowest mean NPA. Because activation of alternative pathways is an important mechanism

Table 2. Univariable and multivariable Cox proportional hazards model for DFS[a]

Variable	Univariable Model			Multivariable Model	
	HR (95% CI)	P		HR (95% CI)	P
Age at surgery, years	1.01 (0.99–1.04)	0.40			
Female vs. male	1.28 (0.81–2.04)	0.29			
Smoking status: ever	0.89 (0.53–1.49)	0.66			
Solid morphologic appearance on preoperative CT	5.93 (2.11–16.68)	0.001		4.39 (1.54–12.49)	0.006
Tumor SUV$_{max}$	1.09 (1.05–1.14)	<0.001		1.07 (1.03–1.12)	0.001
Pathologic stage (8th edition AJCC)					
II vs. I	3.33 (1.88–5.89)	<0.001		1.59 (0.86–2.95)	0.14
III vs. I	7.92 (4.71–13.31)	<0.001		3.92 (2.25–6.81)	<0.001
Pathologic tumor size, cm	1.20 (1.13–1.28)	<0.001			
Lymphovascular invasion	2.51 (1.47–4.28)	0.001			
Predominate histologic subtype[b]	1.41 (0.86–2.33)	0.18			
Visceral pleural invasion	2.76 (1.69–4.50)	<0.001			
Lymph node status: positive	4.90 (3.13–7.66)	<0.001			
Procedure type					
Sublobar vs. lobectomy	1.96 (0.93–4.12)	0.08			
TMB	1.01 (0.99–1.03)	0.26			
FGA	2.13 (0.94–4.84)	0.07			
Number of pathways altered	1.38 (1.12–1.69)	0.002		1.31 (1.06–1.62)	0.01

[a]All models were stratified by pathologic stage except for stage, tumor size, lymph node status, and visceral pleural invasion.
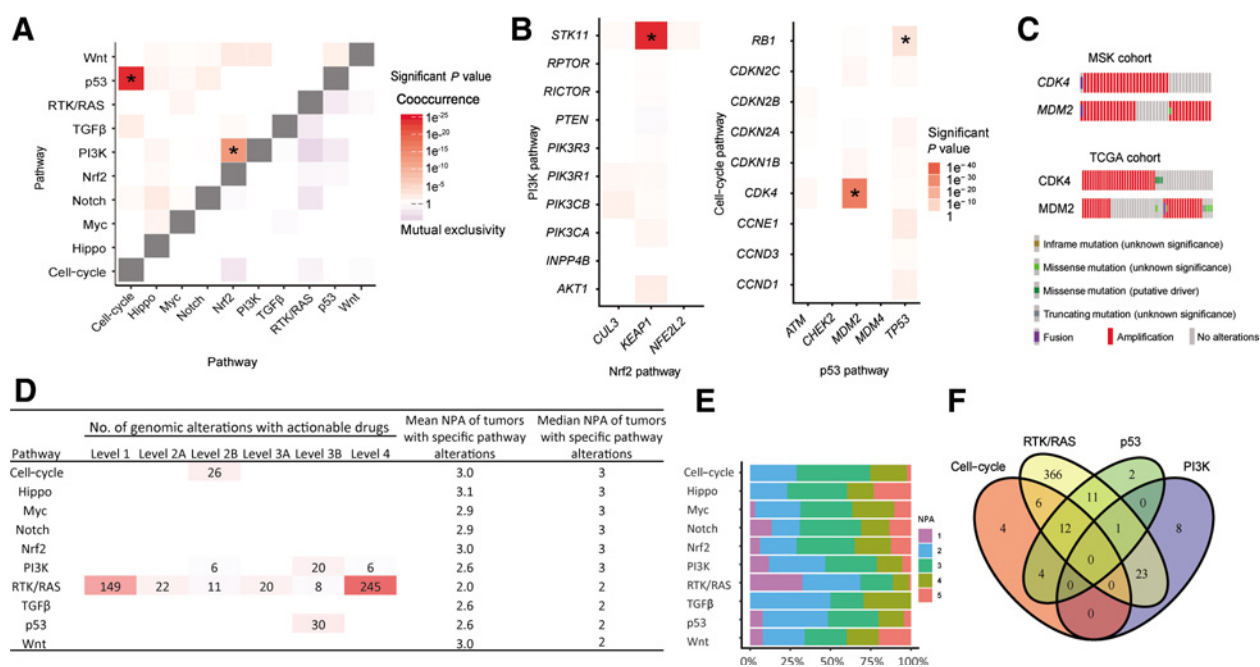[b]Tumor histologic subtypes collapsed as micropapillary + solid versus other subtypes.

Figure 4.
Mutual exclusivity, cooccurrence, and therapeutic actionability. **A,** Cooccurrence and mutual exclusivity between pathways. *, P value is significant ($P < 0.001$, adjusted using the FDR method). Color scale shows the significance of cooccurrence or mutual exclusivity ($-\log P$ value). **B,** Cooccurrence and mutual exclusivity of single genes within PI3K-Nrf2 pathways and p53–cell-cycle pathways. *, P value is significant (*CDK4-MDM2*, $P < 0.001$; *RB1-TP53*, $P = 0.03$; and *STK11-KEAP1*, $P < 0.001$). **C,** Oncoprint of the *CDK4-MDM2*–altered patients in this study and the TCGA pan-cancer atlas cohort. **D,** Actionable genetic alterations within 10 pathways annotated using OncoKB and mean/median NPAs for each pathway. **E,** Stacked bar plot of percentage of NPA (x-axis) in patients with specific pathway (y-axis) altered. **F,** Venn diagram showing patients who have actionable alterations in the four indicated pathways. Intersections of the circles show the number of patients who have >1 actionable pathway.

of acquired resistance to targeted therapy (41), our results suggest the success of drugs in targeting tumors with an isolated RTK/RAS pathway alteration may be secondary to the intrinsically low number of alternative pathways. As *MDM2* and *CDK4* are found in a similar location on chromosome 12q13-15, the amplification of these two genes often occurs together. Using OncoKB to investigate therapeutic actionability, we found that both genes are actionable (level 2B and level 4). This implies the combination of targeted therapies may be effective in patients with alterations in both pathways, which is supported by preclinical studies (42).

Limitations of our study include using DNA sequencing data alone, whereas previous pathway analyses were curated using RNA-seq data (5). Therefore, if a gene is epigenetically silenced through promoter hypermethylation, it would be missed in our analysis. Moreover, the lack of RNA-seq or reverse phase protein array data precludes validation of somatic alterations that can affect the transcriptomic and protein level(s). We also did not evaluate tumor clonality. When two pathways are coaltered, we are unable to distinguish between clonal and subclonal driver mutations. Our ability to assess spatial heterogeneity of pathway alterations over time is limited, as we obtained a single sample per patient. More longitudinal data (i.e., multisampling over time) would allow investigation of the temporal ordering of pathway alterations. We analyzed primarily mitogenic signaling pathways; pathways of other oncogenic processes, such as DNA repair, ubiquitination, and metabolic pathways, were not examined. Most of our cohort is early stage; thus, our results may not be applicable to patients with more advanced stage disease.

Oncogenic pathway analysis using a 10-pathway template characterized by 109 genes from broad-panel NGS revealed important associations with clinicopathologic features. NPA is an independent risk factor for DFS following complete resection of lung adenocarcinoma and is associated with potential therapeutic actionability. Integration of pathway alteration annotation into broad-panel NGS may help stratify patients' risk of recurrence and aid in selection of optimized induction or adjuvant therapeutic strategies.

Disclosure of Potential Conflicts of Interest

V.W. Rusch reports receiving commercial research grants from Genentech and Genelux, is an unpaid consultant/advisory board member for Bristol-Myers Squibb, and reports receiving other remuneration from NIH. N. Rekhtman reports receiving speakers bureau honoraria from Grand Rounds. D.B. Solit is an employee/paid consultant for Pfizer, Loxo Oncology, Lilly Oncology, Illumina, and Vivideon Therapeutics. M.F. Berger is an employee/paid consultant for Roche. D.R. Jones is an employee/paid consultant for Diffusion Pharmaceuticals, Merck, and AstraZeneca. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

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