Lung Adenocarcinoma Biomarker Incidence in Hispanic Versus Non-Hispanic White Patients

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• ***Context.***—**Lung cancer is the leading cause of cancer deaths in the United States and worldwide. Biomarker testing is critical to personalized therapy in lung adenocarcinoma and has been extensively investigated in non-Hispanic whites, Asians, and African Americans. However, little information addresses the underlying genetic changes in lung adenocarcinoma among Hispanic patients in the United States.**

Objective.—**To identify targetable biomarkers other than *EGFR* and *EML4-ALK* in Hispanic patients with lung adenocarcinoma.**

Design.—**We tested DNA extracted from 85 lung adenocarcinoma specimens collected from 40 Hispanic and 43 non-Hispanic white patients for previously reported mutations in *KRAS*, *MET*, *BRAF*, *mTOR*, *STAT3*, *JAK2*, *PIK3CA*, *AKT1 through AKT3*, and *PTEN* with a custom Sequenom massARRAY assay (Sequenom, San Diego, California).**

Results.—**Mutations in *KRAS* were identified in 11 cases (13%; 6 Hispanic [7%], 5 non-Hispanic white [6%]) and had no correlation with sex, age, or smoking history. Mutations in *PIK3CA* were identified in 2 of the 40 Hispanic patients (5%), including one patient (2.5%) with a concurrent *KRAS* mutation. The tumors were wild type for all other genes tested.**

Conclusions.—**Targetable biomarkers other than *EGFR* and *EML4-ALK* were identified in 7 of the 40 Hispanic patients (18%) and 5 of the 43 non-Hispanic white patients (12%), suggesting a similar mutational frequency. Our highly multiplexed genotyping assay detected actionable mutations in 14% (12 of 83) more patients than would have been identified by *EGFR* and *EML4-ALK* testing alone.**

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Lung cancer is the leading cause of cancer deaths in the United States[1] and worldwide.[2] Nevertheless, disease burden is unequally shared among the predominant ethnicities of the US population. According to the 2005–2009 Surveillance, Epidemiology, and End Results database,[3] non-Hispanic whites and African Americans have the highest incidence and mortality due to lung cancer, followed by Asian/Pacific Islanders. Intriguingly, Hispanics have a lower lung cancer incidence and mortality than any of these groups.[4]

Incidence and outcome disparities among patients with lung cancer of different ethnicities are likely multifactorial and compound. However an underlying genetic basis is likely.[5] Oncogenic mutations in *EGFR* have been found in non–small cell lung cancers in only 19% of African Americans[6–9] and 17% of non-Hispanic whites,[10,11] compared with 66% of Asians[10–14] and 33% of Hispanics.[15,16] Furthermore, *EML4-ALK* rearrangements are reported in approximately 6% to 7% of Asians with lung adenocarcinoma, compared with only 1% to 2% of non-Hispanic white patients.[17–20] Targeted therapies are available for both biomarkers, and standard of care requires testing all lung adenocarcinomas for *EGFR* mutations and *EML4-ALK* rearrangements.

Many other driver mutations have been described in non–small cell lung cancer, including *KRAS*, *MET*, *BRAF*, *mTOR*, *STAT3*, *JAK2*, *PIK3CA*, *AKT1* through *3*, and *PTEN*. Therapies targeting each of these biomarkers are currently available and/or in development.[21–23] The incidence of these targetable mutations has been unevenly described among ethnic groups, with a particular paucity of information about lung cancer genetics in Hispanic patients living in the United States. In this study, we investigated 85 lung adenocarcinomas taken from 83 patients, 40 Hispanic and 43 non-Hispanic white, for specific, previously reported mutations with therapeutic agents either available or in development, using a highly multiplexed genotyping assay.

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MATERIALS AND METHODS

With approval by the institutional review board (IRB HSC20110421H), lung adenocarcinoma tumor tissues from 40 Hispanic and 43 non-Hispanic white patients were retrieved from the surgical pathology archives at the University of Texas Health Sciences Center (San Antonio) and the Audie L. Murphy Veterans Affairs Hospital (San Antonio). Demographic information was collected by chart review and included age at diagnosis, sex, stage at diagnosis, and smoking status.

Formalin-fixed, paraffin-embedded tumor tissue was cut in 10-μm sections onto uncharged glass slides. One slide from each case was reviewed by a board-certified pathologist and marked for macrodissection to enrich the sample for tumor cells compared with benign cells. The number of sections used for genomic DNA extraction ranged from 5 to 10 depending on tumor volume. Tissue was deparaffinized using Citrisolv (Fisher Scientific Ireland, Dublin) followed by treatment in 100% ethanol. Puregene cell lysis buffer (Qiagen, Alameda, California) was applied, and tumor tissue was scraped from the slides and digested with proteinase K (Qiagen). The DNA was purified using Qiagen EZ1 tissue kit on an automated workstation according to the manufacturer's instructions. The quantity and quality of genomic DNA (gDNA) was assessed using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, Delaware), and gDNA was stored at –20°C.

Target genes were selected based on a literature search of lung adenocarcinoma biomarkers with therapeutic agents either available or currently in development. These included *KRAS*, *MET*, *BRAF*, *mTOR (FRAP1)*, *STAT3*, *JAK2*, *PIK3CA*, *AKT1* through *AKT3*, and *PTEN* (Table 1). A custom chip to test for these alleles was designed using the MassARRAY Assay Design 3.0 software (Sequenom, San Diego, California). Molecular testing was performed at the Methodist Hospital in Houston, Texas, using a Sequenom MassARRAY instrument (Sequenom). Locus-specific polymerase chain reaction and detection primers were selected, and lung tumor DNA was amplified in a multiplex polymerase chain reaction, followed by a single-base extension reaction. The resulting nucleotides were desalted and transferred to a 384-element SpectroCHIP array, and alleles were discriminated by mass spectrometry.

A χ^2 contingency table analysis was employed to identify significant associations among mutation status and sex, age, ethnicity, smoking history, and stage at diagnosis. Significance was based on a $P < .05$ for the Fisher exact test. There was one case that had only a *PIK3CA* mutation, which was not used in the analyses because of underrepresentation.

RESULTS

Lung adenocarcinoma tumor tissue from 85 specimens representing 83 patients, 40 Hispanic (48%) and 43 non-Hispanic white (52%), was available for testing. Of the 40 Hispanic patients, 5 (13%) were nonsmokers, 13 (33%) were smokers, and 22 (55%) had unknown smoking status. Of the 43 non-Hispanic white patients, 8 (19%) were nonsmokers, 21 (49%) were smokers, and 14 (33%) had unknown smoking status. The 14 Hispanic female patients (35%) were nonsmokers or had unknown smoking status. Among the 12 non-Hispanic white female patients (28%), 2 were smokers and 10 were nonsmokers or had unknown smoking history. Median age at diagnosis among Hispanics was 63 years and was 62 years for non-Hispanic whites.

KRAS mutations were present in 11 (13%) of the 83 lung adenocarcinomas tested, including 6 from the 40 Hispanic patients (15%) and 5 from the 43 non-Hispanic white patients (12%) ($P = .65$; Tables 2 and 3).[24] The *KRAS* mutations among Hispanics included changes to codons 12, 13, and 59, but only to codons 12 and 61 among non-Hispanic whites. Average age among Hispanics with *KRAS* mutations was 67 years; average age among non-Hispanic whites with similar mutations was 65 years. Seven patients (12% of the 57 men tested) with *KRAS* mutations were male and 4 were female (15% of the 26 women tested) ($P = .70$; Tables 2 and 3). Among 11 patients with *KRAS*-mutated tumors, 4 (36%) were smokers, 2 (18%) were nonsmokers, and 5 (45%) had unknown smoking histories ($P = .74$).

PIK3CA mutations were identified in 2 Hispanic (5%) and no (0%) non-Hispanic white patients (Tables 2 and 3). One encoded an E545K amino acid change in an 84-year-old male smoker, and the other was an H1047L change in a 60-year-old woman with unknown smoking status. A concurrent A59T *KRAS* mutation was identified in the second patient.

No mutations in *MET*, *BRAF*, *mTOR (FRAP1)*, *STAT3*, *JAK2*, *AKT1-3*, or *PTEN* were detected.

COMMENT

According to the US Census Bureau, in 2012, approximately 53.3 million Hispanics live in the United States and comprise 17% of the population (n = 313,933,954). By 2060, the US Hispanic population is expected to exceed 128.8 million.[25] Estimates from the 2006–2010 Surveillance, Epidemiology, and End Results database are that Hispanics living in the United States have a lung cancer incidence of 33.5 new diagnoses per 100 000. Thus, more than 17 800 Hispanic patients in the United States can be expected to be diagnosed with lung cancer this year.

Standards of care mandate testing all lung adenocarcinomas for *EGFR* mutations and *EML4-ALK* rearrangements. Many additional biomarkers with associated targeted therapies have been described, and clinical oncologists increasingly recognize these molecularly defined subgroups.[21–23] Reliable information about biomarker frequencies and disparities among ethnic groups is critical, and our study provides key biomarker data about US Hispanics with lung adenocarcinoma.

Two studies have previously reported mutation frequency of *KRAS* codons 12 and 13 in patients with non–small cell lung cancer in Latin America. In the first of these,[15] 650 non–small cell lung cancer specimens from Argentina, Colombia, Peru, and Mexico had a *KRAS* mutation frequency of 16.6% (n = 108). In the second study,[16] *KRAS* mutation frequency among 206 non–small cell lung cancer specimens from Brazilian patients was 14.6% (n = 30). Neither study included analysis of codons 59 and 61, and neither analyzed additional biomarkers implicated in non–small cell lung cancer. The Hispanic population living in the United States includes a considerably more heterogeneous population than may have been represented in previous studies, raising the possibility that *KRAS* mutation frequency among US Hispanics may differ from that reported in Latin America. In addition to increased diversity, our study differed from previous reports by inclusion of *KRAS* codons 12, 13, 59, and 61 and limited patients to those diagnosed with lung adenocarcinoma, rather than the wider spectrum of non–small cell lung cancer.

We discovered that US Hispanic patients with lung adenocarcinoma had a *KRAS* mutation frequency of 15% (6 of 40), consistent with the results from Latin America. Notably, 2 of 40 Hispanic patients (5%) in our study had *KRAS* codon 59 mutations, a finding that would not have been detected in the previous studies. Absent these cases, *KRAS* mutation rate among US Hispanics would have been 10% (4 of 40), suggesting diversity of the US Hispanic

Table 1. Alleles Tested[a]	
Gene	**Alleles Tested**
AKT1	E17K (rs34409589), F35L
AKT2	C574-1G>T, V90L
AKT3	Q124L
BRAF	L597S/R/Q/V V600E/K/R/L G469V/R/S/E/A G466V
JAK2	V617F
KRAS	Composite assays for codon 12 mutations G13V/D A59T Q61E/K/L/R/P/H
MET	T1010I
MTOR	A8S, K42M, L2201L, L888F, M1747L, S1821S
PIK3CA	E542K, E545K, Q546K, H1047R/L, M1043L, S541F
PTEN	G251C, R130G, R233*
STAT3	D661V
STAT4	D661Y
STAT5	G402C
STAT6	N647I
STAT7	Y640F

[a] With Custom MassARRAY Chip on Sequenom Platform, Sequenom, San Diego, California.
* Indicates a premature stop codon.

population might slightly dilute the *KRAS* codon 12 and 13 mutation frequency. Alternatively, the Latin American population may have an even higher *KRAS* mutation frequency than previously reported if changes to codons 59 and 61 are included in future analyses. Furthermore, codon 59 mutations may be more frequent among Hispanics, either those living in the United States or in US and Latin American populations, than it is among non-Hispanic whites, an issue that only larger follow-up studies can resolve.

No meaningful correlation between smoking history and *KRAS* mutation status was identified in our study. Thirty-six of 83 patients (43%) had unknown smoking histories, including 5 of those 36 patients (14%) with *KRAS* mutations, and the absence of smoking history in these cases may have compromised our ability to detect a meaningful correlation.

We identified 2 of the 40 Hispanic patients (5%) with *PIK3CA* mutations. *PIK3CA* is reportedly mutated in 1% to 3% of non–small cell lung cancers, most frequently in squamous cell carcinoma of the lung,[26–29] and is associated with resistance to EGFR tyrosine kinase inhibitors.[27] One of our 83 patients (1%) had concurrent *PIK3CA* and *KRAS* mutations. In a recent study,[30] among 23 patients with

Table 2. Lung Adenocarcinoma Mutations in 40 Hispanic Patients

Case No.	Smoking Status	Age, y/Sex	Stage Grouping at Diagnosis[a]	Mutated Gene	Mutation	Additional Mutated Gene	Mutation
1	Nonsmoker	69/M	IIA	WT			
2	Nonsmoker	75/M	IIB	*KRAS*	A59T		
3	Nonsmoker	53/F	N/A	WT			
4	Nonsmoker	61/F	IA	WT			
5	Nonsmoker	83/M	IB	WT			
6	Smoker	71/M	IIIA	*KRAS*	G12V		
7	Smoker	54/M	IA	WT			
8	Smoker	84/M	IB	*PIK3CA*	E545K		
9	Smoker	72/M	IA	WT			
10	Smoker	73/M	IIIA	*KRAS*	G12D		
11	Smoker	68/M	IIIA	WT			
12	Smoker	80/M	IB	WT			
13	Smoker	79/M	IIIB	WT			
14	Smoker	66/M	IA	WT			
15	Smoker	55/M	IV	WT			
16	Smoker	63/M	IV	WT			
17	Smoker	89/M	IA	WT			
18	Smoker	62/M	IA	WT			
19	Unknown	63/F	IIA	WT			
20	Unknown	55/F	N/A	WT			
21	Unknown	53/F	N/A	WT			
22	Unknown	62/F	IA	WT			
23	Unknown	56/M	N/A	WT			
24	Unknown	55/F	N/A	WT			
25	Unknown	54/F	N/A	WT			
26	Unknown	67/F	N/A	WT			
27	Unknown	49/M	N/A	WT			
28	Unknown	60/F	N/A	*KRAS*	A59T	*PIK3CA*	H1047L
29	Unknown	61/M	N/A	WT			
30	Unknown	60/M	N/A	*KRAS*	G12D		
31	Unknown	59/M	N/A	WT			
32	Unknown	58/M	IA	WT			
33	Unknown	66/F	IA	WT			
34	Unknown	60/F	IIIB	*KRAS*	G13D		
35	Unknown	66/F	IIB	WT			
36	Unknown	63/M	IIB	WT			
37	Unknown	75/M	IA	WT			
38	Unknown	48/F	N/A	WT			
39	Unknown	65/M	N/A	WT			
40	Unknown	69/M	N/A	WT			

Abbreviations: N/A, biopsy only, staging information unknown; WT, wild type.
[a] Staging information per American Joint Committee on Cancer,[24] 2002.

Table 3. Lung Adenocarcinoma Mutations in 43 Non-Hispanic White Patients

Case No.	Smoking Status	Age, y/Sex	Stage at Diagnosis[a]	Mutated Gene	Mutation	Additional Mutated Gene	Mutation
41	Nonsmoker	46/F	IA	WT			
42	Nonsmoker	65/F	N/A	WT			
43	Nonsmoker	60/F	IIIB	*KRAS*	G12V		
44	Nonsmoker	60/F	IB	WT			
45	Nonsmoker	62/F	IIB	WT			
46	Nonsmoker	43/F	N/A	WT			
47	Nonsmoker	79/M	IA	WT			
48	Nonsmoker	88/M	IA	WT			
49	Smoker	48/M	N/A	*KRAS*	Q61L		
50	Smoker	62/F	IA	WT			
51	Smoker	70/M	IA	WT			
52	Smoker	59/M	IIIA	WT			
53	Smoker	49/F	IA	WT			
54	Smoker	50/M	IA	WT			
55	Smoker	61/M	IIB	WT			
56	Smoker	72/M	IA	WT			
57	Smoker	80/M	IA	WT			
58	Smoker	65/M	IIIB	WT			
59	Smoker	64/M	IB	WT			
60	Smoker	53/M	IIIA	WT			
61	Smoker	78/M	IB	WT			
62	Smoker	78/M	IB	WT			
63	Smoker	71/M	IA	WT			
64	Smoker	64/M	IB	WT			
65	Smoker	83/M	IB	*KRAS*	G12V		
66	Smoker	63/M	IA	WT			
67	Smoker	64/M	IB	WT			
68	Smoker	58/M	IIIA	WT			
69	Smoker	63/M	IA	WT			
70	Unknown	35/M	N/A	WT			
71	Unknown	68/M	IB	WT			
72	Unknown	70/M	IA	WT			
73	Unknown	62/M	IB	WT			
74	Unknown	60/M	IB	WT			
75	Unknown	71/F	IA	WT			
76	Unknown	60/M	IA	WT			
77	Unknown	60/F	IV	WT			
78	Unknown	63/F	IIA	WT			
79	Unknown	60/M	IA	WT			
80	Unknown	38/M	IIB	WT			
81	Unknown	61/M	IB	WT			
82	Unknown	55/F	IIIA	*KRAS*	G12A		
83	Unknown	77/M	N/A	*KRAS*	G12V		

Abbreviations: N/A, biopsy only, staging information unknown; WT, wild type.

[a] Staging information per American Joint Committee on Cancer,[24] 2002.

PIK3CA-mutated lung adenocarcinoma, 16 (70%) had a coexisting mutation, and 10 of the 16 (63%) with a coexisting *KRAS* mutation. The remainder (6 of 16; 38%) had other concurrent mutations previously documented to occur at low frequency in lung adenocarcinoma. Patients with coexisting mutations had shorter median survival than did those with isolated *PIK3CA* mutations.

Clinically, identification of coexisting mutations in one patient in our study may have led to therapy directed at the mTOR pathway and to a more-aggressive therapeutic regimen. The current standards of care do not require that testing for lung adenocarcinoma include either of *PIK3CA* or *KRAS* testing, and both mutations would have been missed in directed testing for *EGFR* mutations and *EML4-ALK* translocation. This case highlights the importance of highly multiplexed testing platforms and the mandate for pathologists to pursue assays that detect an array of genetic changes in what are often small tumor samples, including biopsies and fine-needle aspirations.

In conclusion, this study is the first, to our knowledge, to compare the genotype of lung adenocarcinomas in US Hispanics compared with non-Hispanic whites. Our custom, highly multiplexed, genotyping assay found no statistically significant difference in the frequency of targetable biomarkers in lung adenocarcinomas in Hispanics compared with non-Hispanic whites. These findings do not explain the lower frequency and better survival of US Hispanic patients with lung cancer compared with other ethnicities. However, our findings do underscore the need to test all patients newly diagnosed with lung adenocarcinoma, regardless of ethnicity.

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