Frequency of *EGFR* and *KRAS* Mutations in Lung Adenocarcinomas in African Americans

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Introduction: The detection of mutations in the epidermal growth factor receptor (*EGFR*) gene, which predict sensitivity to treatment with *EGFR* tyrosine kinase inhibitors, represents a major advance in the treatment of lung adenocarcinoma. *KRAS* mutations confer resistance to *EGFR*-tyrosine kinase inhibitors. The prevalence of these mutations in African American patients has not been thoroughly investigated.

Methods: We collected formalin-fixed, paraffin-embedded material from resected lung adenocarcinomas from African American patients at three institutions for DNA extraction. The frequencies of *EGFR* exon 19 deletions, exon 21 L858R substitutions, and *KRAS* mutations in tumor specimens from African American patients were compared with data in white patients ($n = 476$).

Results: *EGFR* mutations were detected in 23 of the 121 specimens from African American patients (19%, 95% confidence interval [CI]: 13–27%), whereas *KRAS* mutations were found in 21 (17%, 95% CI: 12–25%). There was no significant difference between frequencies of *EGFR* mutations comparing African American and white patients, 19% versus 13% (61/476, 95% CI: 10–16%; $p = 0.11$). *KRAS* mutations were more likely among whites, 26% (125/476, 95% CI: 23–30%; $p = 0.04$).

Conclusions: This is the largest study to date examining the frequency of mutations in lung adenocarcinomas in African Americans. Although *KRAS* mutations were somewhat less likely, there was no difference between the frequencies of *EGFR* mutations in African American patients, when compared with whites. These results suggest that all patients with advanced lung adenocarcinomas should undergo mutational analysis before initiation of therapy.

Key Words: *EGFR* mutation, *KRAS*, African Americans, Racial differences.

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The discovery of activating mutations in the epidermal growth factor receptor (*EGFR*) gene has revolutionized our understanding and treatment of lung adenocarcinoma, a disease diagnosed in 120,000 Americans each year.[1] Somatic mutations in the tyrosine kinase domain of the *EGFR* gene drive the development of a substantial subset of lung adenocarcinomas and sensitize these tumors to treatment with the small molecule tyrosine kinase inhibitors (TKIs) erlotinib and gefitinib.[2–4] When patients with *EGFR* mutations are treated with *EGFR*-TKIs, nearly all respond, with improvement in progression-free survival compared with patients without *EGFR* mutations.[5] As a result, there has been significant interest in molecular characterization of *EGFR* and in identification of patients with *EGFR* mutations.

Approximately one third of lung adenocarcinomas worldwide and an estimated 50% of tumors from East Asian patients have *EGFR* mutations. Nevertheless, only 15% of patients from an unselected North American population will harbor these mutations.[6] The prevalence of *EGFR* mutations among patients of other races, such as African Americans, is unknown, in part due to small numbers of patients included and/or reported in prior studies.

KRAS mutations are also of interest in patients with lung adenocarcinomas. Transversion mutations (substitution of a pyrimidine for a purine or purine for a pyrimidine) are more common than transition mutations (substitutions of a purine for a purine or pyrimidine for a pyrimidine). *KRAS* mutations and *EGFR* mutations are mutually exclusive, and

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the presence of a *KRAS* mutation identifies a group of patients who are unlikely to respond to an *EGFR*-TKI.[7–9]Although *EGFR* mutations are found more typically in never smokers, *KRAS* mutations were first identified among patients who reported heavy tobacco use. More recently, however, *KRAS* mutations have also been identified in 15% of never smokers, when compared with 22% of former smokers in one study from our institution.[10] *KRAS* mutations are found in 20 to 30% of patients with lung cancer in North American populations, and in Asian populations, they are less frequently reported. The true prevalence of *KRAS* mutations among African Americans is unknown.

In a prior study of 291 tumors obtained from patients from Memorial Sloan-Kettering Cancer Center (MSKCC), 6 of 14 African Americans (43%) harbored *EGFR* mutations in their tumors.[11] On the basis of this higher than expected frequency, we aimed to study the rate of both *EGFR* and *KRAS* mutations in the largest cohort of African American patients with lung adenocarcinoma to date.

METHODS

Tumor specimens were obtained from all available tissue from African American patients from two institutions MSKCC (New York, NY) and SUNY Downstate Medical Center (Brooklyn, NY) and population-based cases from studies conducted at Wayne State University (Detroit, MI). African American patients were selected based on self-report of race, as determined at the time of registration. The histologic diagnosis of adenocarcinoma was verified using the pathology report provided by participating institutions.

Approval from the MSKCC Institutional Review Board/Privacy Board was obtained for the acquisition and analysis of all tumor specimens. All mutational analysis was performed in the Department of Pathology at MSKCC from formalin-fixed, paraffin-embedded material. Tumor specimen only underwent microdissection before mutational analysis when necessary. *EGFR* exon 19 deletions and exon 21 L858R mutations were identified using previously reported mutation-specific polymerase chain reaction-based methods.[12,13] *KRAS* codon 12 and codon 13 mutation identification was performed by both mass spectrometry (Sequenom)-based genotyping and direct sequencing. All positive cases were identified by visual review and independently confirmed by direct sequencing.

EGFR and *KRAS* mutation frequencies were calculated and compared with mutation frequencies of surgically resected lung adenocarcinomas collected from white patients evaluated at MSKCC between 2006 and 2008. Mutational analysis of these tumors was also performed by the Department of Pathology.

The frequency of *EGFR* and *KRAS* mutations was summarized and compared using χ^2 tests and 95% confidence intervals (CIs).

RESULTS

One hundred twenty-one lung adenocarcinomas from African American patients were collected—approximately equal numbers from MSKCC ($n = 50$) and Wayne State University ($n = 47$), with additional samples from SUNY Downstate Medical Center ($n = 24$). No other clinicopathologic data were collected for this analysis.

EGFR exon 19 deletions or exon 21 L858R mutations were identified in 23 (19%) of the 121 African American specimens (95% CI: 13–27%). Among the *EGFR* mutations, 18 had exon 19 deletions and five had exon 21 L858R mutations. *KRAS* exon 2 codon 12 and 13 mutations were identified in 21 (17%, 95% CI: 12–25%) of the 121 African American tumor specimens (Table 1).

To evaluate mutation rates by race, we compared the data collected from African American patient samples to mutation frequencies of all lung adenocarcinomas resected from white patients between 2006 and 2008 at MSKCC, a total of 476 patients (Figure 1). The prevalence of *KRAS* mutations in African American patients was less than that observed in the white group (26%, 125/476, 95% CI: 23–30%; $p = 0.04$). Nevertheless, there was no difference in the number of *EGFR* mutations between African American and white patients (13%, 61/476, 95% CI: 10–16%; $p = 0.11$).

Transversion mutations were demonstrated in 17 (81%, 95% CI: 59–93%) of the African American patients harboring *KRAS* mutant lung cancers, and only four (19%, 95% CI: 7–41%) had transition mutations. Similarly, 87 (70%, 95% CI: 62–78%) of *KRAS* mutations in the white cohort were transversions, whereas 37 (30%, 95% CI: 22–38%) were transitions ($p = 0.44$). The distribution of *KRAS* mutations and their frequencies in both cohorts are listed in Table 2. The most common type of *KRAS* mutation found in both cohorts was the glycine to cysteine transversion (G12C). The least common *KRAS* mutations were G13V, G12R, G12S, and

TABLE 1. Distribution and Frequency of Mutations Among African Americans

Mutations Identified	African Americans ($n = 121$)	95% CI (%)
EGFR exon 19 deletion, *n* (%)	18 (15%)	10–22
EGFR L858R exon 21 mutation, *n* (%)	5 (4%)	2–10
KRAS mutations, *n* (%)	21 (17%)	12–25
EGFR/KRAS wild type, *n* (%)	66 (55%)	46–63

CI, confidence interval; *EGFR*, epidermal growth factor receptor.

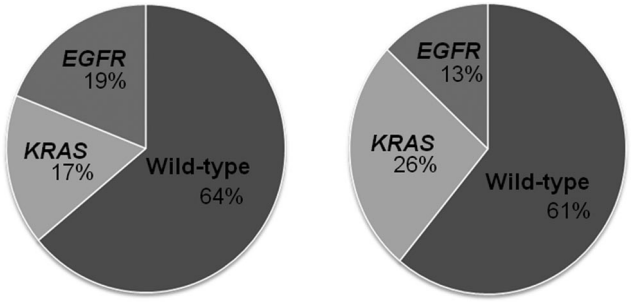

FIGURE 1. Frequencies of *EGFR* and *KRAS* mutations among African American and white populations.

TABLE 2. Distribution of *KRAS* Mutations Among Whites and African American Patients

KRAS Mutation	*KRAS* Nucleotide	African American Patients ($n = 21$)	White Patients ($n = 125$)	p
G12C	GGT→TGT	8 (38%)	48 (38%)	1
G12V	GGT→GGT	7 (33%)	18 (14%)	0.0547
G12A	GGT→GCT	1 (5%)	13 (10%)	[a]
G13C	GGC→TGC	1 (5%)	7 (6%)	[a]
G13V	GGC→GTC	0 (0%)	1 (1%)	[a]
G12R	GGT→CGT	0 (0%)	1 (1%)	[a]
G12D	GGT→GAT	3 (14%)	32 (26%)	[a]
G13D	GGC→GAC	0 (0%)	4 (3%)	[a]
G12S	GGT→AGT	0 (0%)	1 (1%)	[a]
G13S	GGC→AGC	1 (5%)	0 (0%)	[a]

[a] Numbers are too small for meaningful comparison.
AA, African American.

G13S. Of these, only G13S was not represented in the larger white group.

DISCUSSION

This study represents the largest retrospective study to date comparing *EGFR* and *KRAS* mutation rates between African American and white populations. There was no difference in the frequency of *EGFR* mutations between the African American and white cohorts, whereas *KRAS* mutations were less frequent among African American patients.

A few small studies have previously evaluated the prevalence of mutations by race. In one study performed in Louisiana, the frequency of *KRAS* mutations was evaluated in 116 patients with lung cancer, 60 of them were African Americans.[14] In contrast to our findings, African American patients in that study were significantly more likely to harbor a *KRAS* mutation, when compared with white patients (37% versus 20%, $p = 0.048$). Nevertheless, consistent with our data and reports by others, Hunt et al.[14] also reported a "disproportionately high number of cysteine for glycine transversions." Cysteine missense substitutions result from a G→T transversion in the first base in either codon 12 or 13. They have been attributed to the polycyclic aromatic hydrocarbons present in tobacco smoke.[15] Hunt et al.[14] also found a higher than expected number of serine mutations among the African American cohort as the result of a G→A transition in the first base of either codon 12 or 13. This mutation was demonstrated in one African American patient in this study and one patient in our white cohort (Table 2).

Racial differences in *EGFR* mutation rates have also been evaluated in smaller numbers in prior studies (Table 3). *EGFR* mutations were reported in 1 of 44 African American patients (2.4%) in a study of 219 patients with tissue specimen from the University of Maryland, the Mayo Clinic in Minnesota, and the University of Milan, Italy.[16] In comparison, 25 of the 177 (14.1%) tumors from white patients harbored *EGFR* mutations. Another study from the University of Texas M. D. Anderson Cancer Center included only eight African American patients.[17] Although 14 *EGFR* mutations were confirmed among the 159 patients included, none were reported in this small subset of African American patients. Although *KRAS* mutations were also reported in 18 patients overall, their races were not specified.

Most recently, Leidner et al.[18] published an analysis of 53 tumors from African American patients obtained from the University Hospitals Case Medical Center in Cleveland, OH. Patients were selected based on the availability of a tissue block and uninvolved paired tissue (i.e., from a lymph node). Results were compared with a white cohort of 121 patients from three separate Italian centers (Bologna, Milan, and Perugia) previously enrolled in an unrelated clinical trial to receive gefitinib, who had mutational analysis performed previously. In this study, African Americans were again less likely to have *EGFR* mutations in their tumors, when compared with white patients (2% versus 17%, $p = 0.022$). Nevertheless, the only *EGFR* mutation found among the African American patients was neither of the common activating *EGFR* mutations (exon 19 deletion or exon 21 L858R substitution) but a missense mutation in exon 20 L768N, a mutation in *EGFR* not previously reported. Leidner et al. also reported no difference in *KRAS* mutation frequencies between the two cohorts of patients.

Twice the number of African American patients ($n = 121$) were included in our analysis compared with 53 tumors from African American patients tested in the article of Leidner et al. Although not as robust a sample as our white cohort, a retrospective power calculation indicates that we have adequate power to detect a difference of 9.6% or more in either direction, assuming the frequency of EGFR mutations in the white population is 13%. Therefore, if it were true that *EGFR* mutations exist among African American patients at a much lower frequency, as reported in

TABLE 3. Prior Studies Comparing the Frequencies of EGFR Mutations in African American and White Patients

Study	African American Patients	White Patients	Frequency *EGFR* Mutations, African American Patients, *n* (%)	Frequency *EGFR* Mutation, White Patients, *n* (%)
Yang et al.[16]	41	177	1 (2.4), 95% CI: 0–12.9%	25 (14%), 95% CI: 9.4–20.1%
Tsao et al.[17]	8	139	0 (0), 95% CI: 0–40%	10 (7%), 95% CI: 4–13%
Leidner et al.[18]	53	102	1 (2), 95% CI: 0–11%	15 (17%[a]), 95% CI: 11–26%
Reinersman et al. (this study)	121	476	23 (19), 95% CI: 13–27%	61 (13%), 95% CI: 10–16%

[a] Eighty-nine of 102 whites analyzable for EGFR mutations.
CI, confidence interval; *EGFR*, epidermal growth factor receptor.

previous studies (Table 3), we would have detected that difference in our analysis.

One potential source of bias in our study is the fact that African American specimens were collected from three different centers, whereas white patients were collected only from MSKCC. Similar to the study by Leidner et al. described earlier, patients were included based solely on tissue availability, a common criterion for translational tissue studies. No other sample selection was used, and because all African American patients who had available tissue were included, we expect the study population to be representative of the African American population of each participating institution. An analysis restricted to MSKCC patients found 16% of African Americans had *EGFR* mutations, and 25% had *KRAS* mutations, both safely within the 95% CIs for the total number of African American patients included in the results.

We did not report clinical characteristics traditionally associated with response to EGFR-TKIs in our patients. Before the IRESSA Pan-Asian Study, we relied on histology, ethnicity, and smoking status to predict the likelihood of response to EGFR-TKI treatment. Nevertheless, we now know that it is the presence of the *EGFR* mutation that truly underlies sensitivity. The most meaningful demonstration of this principle from IPASS was the paltry response (1%) of Asian women never smokers or former light smokers randomized to receive gefitinib who were found to be without EGFR mutations in their tumors.[5] Testing tumors for the presence of *EGFR* and *KRAS* mutations before treatment with the EGFR-TKI gefitinib is now a standard of care in Europe. This standard will increasingly be adopted on all continents, and therefore, attention to clinical characteristics for the purposes of predicting response to EGFR-TKIs will continue to lessen in importance.

Our data indicate that lung cancers arise from *EGFR* mutations in African Americans just as commonly as in white patients. Therefore, African American patients stand to derive similar benefit from treatment with *EGFR*-TKIs as other western populations. We believe that these results have important diagnostic and treatment implications for African American patients and any non-Asian, non-white patients with lung cancer. Irrespective of race, tumors tissue obtained from all patients with advanced lung adenocarcinoma should be screened for the presence of *EGFR* and *KRAS* mutations. Future research efforts examining other molecular mechanisms, such as *MET* germline polymorphisms,[19] *BRAF* mutations, *HER2* mutations, and *ALK* translocations, may further elucidate the racial variations in lung cancer development.

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