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Cancer Genes in Lung Cancer: Racial Disparities: Are There Any?

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Abstract

Cancer is now known as a disease of genomic alterations. Mutational analysis and genomics profiling in recent years have advanced the field of lung cancer genetics/genomics significantly. It is becoming more accepted now that the identification of genomic alterations in lung cancer can impact therapeutics, especially when the alterations represent "oncogenic drivers" in the processes of tumorigenesis and progression. In this review, we will highlight the key driver oncogenic gene mutations and fusions identified in lung cancer. The review will summarize and report the available demographic and clinicopathological data as well as molecular details behind various lung cancer gene alterations in the context of race. We hope to shed some light into the disparities in the incidence of various genetic mutations among lung cancer patients of different racial backgrounds. As molecularly targeted therapy continues to advance in lung cancer, racial differences in specific genetic/genomic alterations can have an important impact in the choices of therapeutics and in our understanding of the drug sensitivity/resistance profile. The most relevant genes in lung cancer described in this review include the following: *EGFR, KRAS, MET, LKB1, BRAF, PIK3CA, ALK, RET*, and *ROS1*. Commonly identified genetic/genomic alterations such as missense or nonsense mutations, small insertions or deletions, alternative splicing, and chromosomal fusion rearrangements were discussed. Relevance in current targeted therapeutic drugs was mentioned when appropriate. We also highlighted various targeted therapeutics that are currently under clinical development, such as the MET inhibitors and antibodies. With the advent of next-generation sequencing, the landscape of genomic alterations in lung cancer is expected to be much transformed and detailed in upcoming years. These genomic landscape differences in the context of racial disparities should be emphasized both in tumorigenesis and in drug sensitivity/resistance. It is hoped that such effort will help to diminish racial disparities in lung cancer outcome in the future.

Keywords:

lung cancer, racial disparities, cancer gene, targeted therapy

Introduction

Lung cancer is one of the most common human cancers and represents the leading cause of cancer mortality in the United States (US). There were an estimated 221,130 new cases and 156,940 deaths in 2011.[1] The overall survival rate of lung cancer patients remains poor despite available standard treatments. Recent advances in the fields of mutational analysis and molecularly targeted therapy made it possible to develop new receptor kinase inhibitors such as erlotinib and gefitinib (against epidermal growth factor receptor [EGFR]) and most recently crizotinib (against rearranged anaplastic lymphoma kinase [ALK]) and antibodies such as cetuximab (against EGFR) and bevacizumab (against vascular endothelial growth factor [VEGF]).[2] These discoveries yielded better response rates and have marked a new era of paradigm change in targeted lung cancer personalized therapy. Moreover, in recent years, lung cancer molecular profiling has been largely fueled by the tremendously fast scientific and technological advancement in cancer genome research with high-output genomic analysis platforms. Mutational cancer gene analysis has shifted from single gene analysis and later gene family analysis to more recently high-throughput next-generation global genome sequencing analysis, including sequencing of the whole cancer genome, exome, transcriptome, or epigenome. The vast amount of genomic information in a generation is expected to transform our current understanding of lung cancer and would in turn usher a new era of personalized lung cancer therapy. In fact, a number of institutions have already begun to integrate molecular profiling and even clinical next-generation sequencing (NGS) into routine lung cancer diagnosis to empower treatment decisions.[3]

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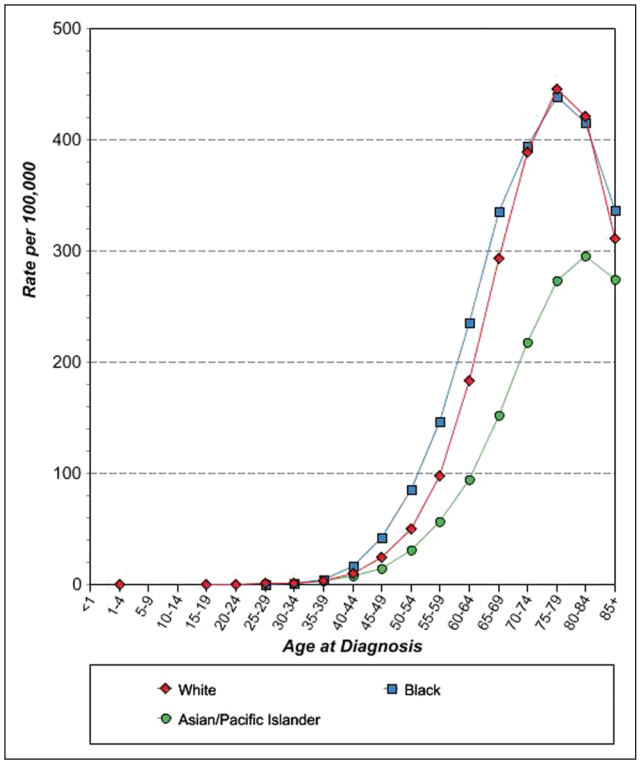

Figure 1. Age-specific SEER lung cancer incidence rates in the United States: 2000-2008.
Fast Stats: An interactive tool for access to SEER cancer statistics. Surveillance Research Program, National Cancer Institute. http://seer.cancer.gov/faststats.

The increasingly growing genetic and genomic database of lung cancer now sheds light into the racial differences of lung cancer genetics across different human populations. These findings may translate into an important force in the development of treatment algorithm and therapeutic choices in the new era of genomics-guided lung cancer personalized medicine. Nonetheless, the full spectra of the disparities in lung cancer genetics among different human populations are still lacking for the most part. In this review, we attempt to provide a concise summary of the current understanding and available data on the racial differences of lung cancer genes among different human populations.

Race and Lung Cancer Outcome Disparities among Different Populations

Health disparities in the US are a recognized phenomenon that has been well documented in the literature (Fig. 1). Discrepancies between African American and white lung cancer patient populations are supported by data from the 2000-2008 Surveillance, Epidemiology, and End Results (SEER) database.[4] It exhibits substantial disparities: the average annual age-adjusted incidence for lung cancer between 2000 and 2008 was 75.2 per 100,000 for African Americans and 64.9 per 100,000 for whites (data from the SEER 17 Registry database for 2000-2008). Notably, African American lung cancer patients were less likely to receive surgical treatment compared to their white counterparts. Women, older patients, and patients in lower socioeconomic classes displayed significantly lower rates of surgery compared to patients in higher socioeconomic classes.[4] Further evidence from SEER 17 data using age-specific analyses shows that African Americans under the age of 50 years are almost twice as likely to develop lung cancer as whites in the same age group. The mean age at diagnosis was 66 years in African Americans compared with 71 years in whites.[5] In the age group between 40 and 54 years, African American men are 2 to 4 times more likely to develop lung cancer than white men after adjusting for smoking history.[6] Health outcome disparities among diverse racial groups in the US are multifactorial and compound. Although socioeconomic and cultural differences across racial groups can account for some of the current disparities, recent evidence from the evolving field of lung cancer genetics research is beginning to transform the way we address these differences in health outcomes from a macroscopic intervention into an increasingly personalized molecular approach.

Lung Cancer: Genes and Genome

Copy Number Variations. Copy number alterations in lung cancer have been studied using dense single-nucleotide polymorphism (SNP) arrays, providing us with further insight about the molecular basis of the disease. Weir *et al*.[7] identified 57 significantly recurrent events from a cohort of 371 tumors. The most commonly identified event was chromosome 14q13.3 amplification, accounting for 12% of all the tumor samples. A novel proto-oncogene involved in a significant fraction of lung adenocarcinomas was identified as *NKX2*-1 (NK2 homeobox 1, also called *TITF1*), which resides in the 14q13.3 amplification interval and encodes a lineage-specific transcription factor. Interestingly, a recent study examining the TITF1 protein and genomic expression in non–small cell lung cancer (NSCLC) using integrative immunohistochemistry (for protein expression), FISH, and qPCR (for gene copy number) analysis revealed that the protein versus genomic patterns of TITF1 have opposing roles in NSCLC prognosis and may occur preferentially in different subsets of NSCLC patients with distinct oncogenic mutations.[8] Broet *et al*.[9] reported significantly higher rates of copy number gain on 16p13.13 and 16p13.11 in East Asian patients' tumor samples while higher rates of genomic loss on 19p13.3 and 19p13.11 occurred in white patients. A novel oncogene *FUS* was found to be frequently associated with gain in copy number in the 16p region in lung adenocarcinoma in never smokers in addition to the

finding of *MYC* gene copy number gain.[10] Both *EGFR* and *KRAS* gene copy number gains have been found to occur more frequently in tumors harboring the activating mutations of the respective oncogene.[11]

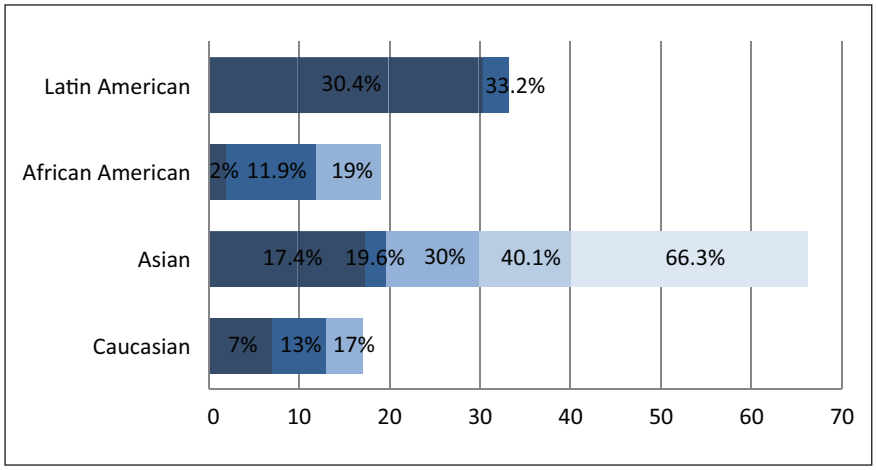

Figure 2. Spectrum of *EGFR* oncogenic driver mutations among different racial groups with NSCLC. The different color shades represent *EGFR* mutational rates reported by different studies. Data on the African American and Latin American cohorts are based on a limited number of studies available.[46,55-58] Data on the Asian and white cohorts are abundant over recent years, and several representative studies were selected for graphical representation here.[23,24,28,46,56,101,143]

Mutations. The unprecedented advances in lung cancer genome analysis in recent years have revolutionized our understanding of the disease at a deeper molecular scale. First, the analysis of entire gene families (e.g., protein kinome, lipid kinome, and tyrosine phosphatome) as part of DNA mutational profiling of cancer genes in lung cancer unveiled vital information about the molecular structure of the disease. Protein mutations of the RAS/RAF/MEK/MAP kinase signaling pathway were studied in the first of its kind large-scale system.[12] The study showed that serine/kinase BRAF was frequently mutated in human cancer at a frequency of 66% in malignant melanoma and at a less dramatic rate in other types of cancer including lung cancer (2% in primary adenocarcinoma). The discovery of cancer-associated mutations was driven by systemic resequencing of the cancer genome. A recent study intending to discover new somatic mutations in 188 human lung adenocarcinomas[13] revealed over 1,000 somatic mutations after DNA sequencing of 623 genes with known or suspected cancerous activity. It identified 26 genes with a significantly high mutagenesis rate, possibly implicating them in tumorigenesis. Other frequently mutated genes include tyrosine kinases such as EGFR homolog ERBB4 and multiple Ephrin receptor genes such as EPHA3, VEGFR2 (KDR), and NTKR. These studies provide us with insight into key signaling pathways in lung adenocarcinoma tumorigenesis, which can serve as novel molecular targets for future therapeutic development.

In the following, we will provide a review with emphasis on the molecular genetic variations in several key molecular targets that are documented in lung cancer literature (*EGFR*, *BRAF*, *KRAS*, *MET*, *LKB1*, and *PIK3CA*). The review was focused also on the racial differences of these cancer genes among human populations and the implication of such differences in the future of personalized cancer therapy.

EGFR (HER1 or ERBB1). EGFR is the key paradigm of molecular targeted therapy in lung cancer, which is now commonly used in the clinical setting worldwide (Fig. 2). Current available drugs that target EGFR can be divided into 2 categories: small-molecule EGFR tyrosine kinase inhibitor (TKI)—gefitinib and erlotinib—and monoclonal anti-EGFR antibody—cetuximab. The genomic discoveries in *EGFR* and the resultant targeted treatment opened a new window of opportunity for our renewed understanding in lung cancer biology and therapy. This paradigm shift has mainly been fueled by study findings, which reported that a specific cluster of *EGFR* gene mutations in lung adenocarcinoma resulted in enhanced sensitivity and clinical response to EGFR kinase inhibitors gefitinib[14,15] and erlotinib.[16] The *EGFR* mutation database massively grew following research efforts covering thousands of patient tumor samples. *EGFR* mutations that exist in NSCLC were found to be predominantly somatic, while only a few including T790M were found to be germline in nature. Exons 18 to 21 within the tyrosine kinase domain were the most heavily sequenced region, as it is considered to harbor the mutational hot spots. There are also other *EGFR* mutations that reside outside these hot spot exons, some having a unique impact on TKI sensitivity, albeit occurring at a relatively lower frequency; for example, the E884K mutation in exon 22 is more sensitizing to gefitinib but confers insensitivity to erlotinib.[17,18] The majority (85%) of the currently identified *EGFR* kinase mutations can be attributed to the L858R missense mutation in exon 21 and short in-frame deletion variants in exon 19,[19,20] both being found sensitizing to EGFR TKIs. Several reports suggest that *EGFR* mutations may carry a prognostic value.[21,22] Exon 19 deletions and L858R were found to exist in a subset of NSCLC patients with unique clinical characteristics. These patients were usually never or light female smokers with an adenocarcinoma histology.

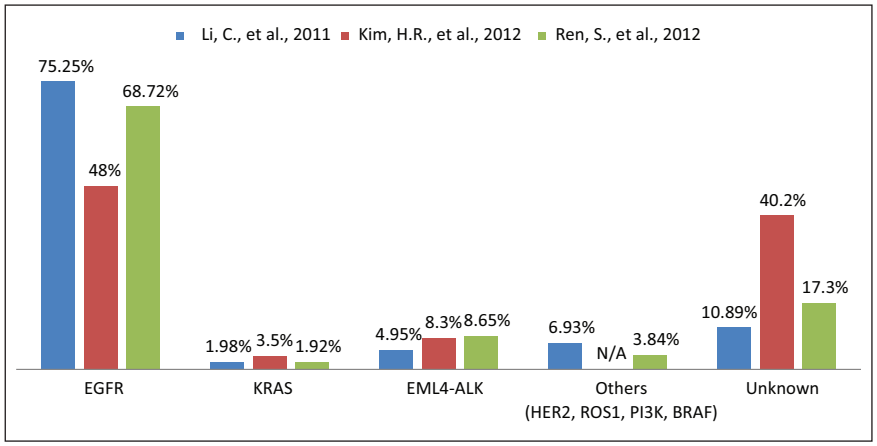

Figure 3. Spectrum of oncogenic driver mutations in Asian never smokers with lung adenocarcinoma.[31-33] The data were collected from 3 different studies to represent the mutational frequency range of different genes among the same population. N/A = not available.

Interestingly, the frequency of *EGFR* mutations differed among different racial groups in the population. *EGFR* mutations were highly prevalent in the Asian patient population at 30% compared to only 7% in whites.[23] Other studies frequently reported similar *EGFR* mutational frequencies, ranging from 19.6% to 40.1% among different ethnicities within the Asian population.[24-30] The *EGFR* mutational frequency was found to be even higher among Asian never smokers at a rate range between 48% to 75.3%[31-33] (Fig. 3). In 2009, the IRESSA Pan-Asia Study (IPASS) results, which compared gefitinib with carboplatin-paclitaxel, were reported. The trial was conducted on never or light smokers of Asian descent with adenocarcinoma (higher frequency of sensitizing *EGFR* mutations) as a mutation-enriching strategy for the study population. The trial demonstrated that the gefitinib-treated group had a 12-month progression-free survival rate of 24.9% compared to 6.7% in the chemotherapy-treated group.[34] It was concluded that the presence of *EGFR* mutations was a strong predictive factor for improved outcome with gefitinib therapy as a first-line therapy. Remarkably, patients with no identifiable *EGFR* mutations, despite possessing the clinical parameters of higher probability of having the mutations, in fact developed a worse outcome with gefitinib therapy. Other subsequent phase 3 studies in different parts of the world using both gefitinib and erlotinib tested in the first-line therapy setting to compare with cytotoxic chemotherapy further substantiated the paradigm of first-line EGFR TKI use among patients proven to harbor the EGFR-sensitizing mutations in a genotype-informed targeted therapy paradigm.[35]

EGFR gene copy number has also been intensely investigated in lung cancer. It was found to be increased in 30% to 50% of NSCLC patients.[36] Accordingly, some studies reported that *EGFR* gene copy number may play a predictive role in EGFR TKI therapy response.[37,38] Nevertheless, other studies reported that these earlier findings were confounded by the fact that a high *EGFR* copy number can significantly co-exist with *EGFR* mutations. Furthermore, differences in assay platforms for gene copy numbers such as FISH and genomic real-time qPCR, in addition to tissue quality variations, can further confound the results and their interpretations. With the growth of EGFR TKI prescription use in the lung cancer clinic, clinical acquired resistance became inevitable. The predominant resistant mechanism was found to be *EGFR* T790M mutation in exon 20, which is part of the hydrophobic "gatekeeper" residue within the kinase domain, which confers resistance to both gefitinib and erlotinib.[39-41] The T790M mutation accounts for almost half the cases of acquired resistance.[42] Other documented resistance cases were associated with primary resistance mutations such as exon 20 insertions (D770_N771 insNPG)[43] and acquired resistance mutations such as exon 19 missense mutation D761Y.[44,45] It is noteworthy that N771GY and A767-V769dup, which are novel somatic insertion mutations in exon 20, and S768N, a missense mutation, were all identified in the African American cohort.[46,47] In East Asian patients, S768I, a somatic EGFR mutation, was reported with evidence, suggesting a potential role in EGFR TKI resistance.[24,27,48,49] Yet, these acquired genetic resistances were not exclusive to any racial group. *MET* genomic amplification has also been reported to be implicated in acquired EGFR TKI resistance.[50,51] More novel mechanisms of acquired resistance have emerged from larger tumor rebiopsy studies, which also include *PIK3CA* mutation, *EGFR* amplification, and small cell lung cancer (SCLC) transformation.[52]

Although abundant research data were collected on the frequency of *EGFR* mutations in the Asian and white populations, corresponding information regarding the African American population remains deficient. Health disparities exist between African Americans and the rest of the US population, resulting in inferior health outcomes.[5,53] The prevalence of *EGFR* mutations in the African American population varies significantly in the documented literature. In one study by Yang *et al.*,[54] *EGFR* mutations were found in only 2.4% of African Americans compared to 14.1% in whites. Another supporting study found that only 2% of

African American patients expressed activating *EGFR* mutations versus 17% in whites.[46] Yet, in a recent study by Cote *et al*.,[55] it was found that *EGFR* mutations existed in 11.9% of NSCLC tumors of African American patients versus 15.6% in whites, that is, more comparable frequencies. This finding was supported by Reinersman *et al*.,[56] who reported *EGFR* mutations in 19% of the African American NSCLC samples versus 13% in whites, arguing that all patients with advanced lung adenocarcinoma should undergo mutational analysis before the initiation of therapy. Interestingly, in 2 recent studies conducted in a Latin American cohort, the *EGFR* mutational frequency was significantly higher at 30.4% to 33.2%.[57,58]

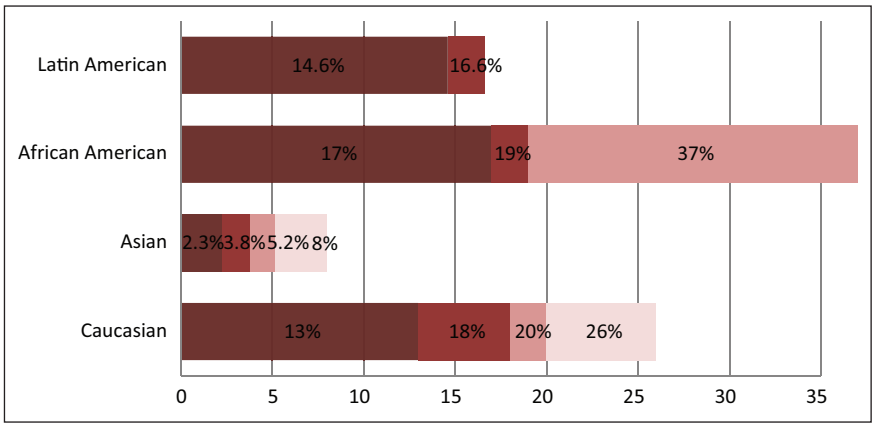

Figure 4. Spectrum of *KRAS* oncogenic driver mutations among different racial groups with NSCLC. The different color shades represent *KRAS* mutational rates reported by different studies. Data on the African American and Latin American cohorts are based on a limited number of available studies.[46,56-58,73] Data on the white cohort are based on multiple studies including 2 meta-analyses of 22 studies with 1,470 NSCLC patients.[23,46,56,71-73] Data on the Asian cohort are based on studies conducted in the Chinese and Korean populations.[143-145]

The *EGFR* mutational status seems also to vary between the primary lung tumor and the corresponding metastases. Often, the *EGFR* mutations would be present in the primary lung tumor but appear to be absent in the metastases. According to several studies, the discordance rate of *EGFR* mutations ranged between 16.2% to 32.5%.[59-62]

Emerging clinical data in studies testing a molecularly matched targeted therapy approach particularly in mutation-enriching patient populations using clinicopathological parameters, for example, the IPASS, have now strengthened the notion that molecular tumor selection by profiling trumps clinical selection.[63] The IPASS also paved the road for the arrival of the first-line use of EGFR TKI (erlotinib and gefitinib) in sensitizing EGFR mutation–positive advanced NSCLC patients. Going forward, this position is likely to be even more strengthened by emerging genomic analysis of the lung cancer genome among different populations. The declining cost of high-throughput tumor molecular profiling would also facilitate and further justify this approach of genotype-informed therapy decision in lieu of "clinical profiling" or "racial profiling" for therapy decisions.

KRAS. *KRAS* encodes a GTPase that plays the role of a central mediator of downstream growth factor receptor signaling and therefore is critical for cell proliferation, survival, and differentiation (Fig. 4). *KRAS* gene mutations are uncommon in squamous cell carcinoma but can be present in approximately 15% to 25% of lung adenocarcinomas.[64] The mutations are missense mutations primarily in codons 12 and 13 of (exon)[1]. In the vast majority of cases, *KRAS* mutations were found in *EGFR* wild-type tumors; hence, *EGFR* and *KRAS* mutations were mutually exclusive.[65-67] Although mutant KRAS has well-established poor prognostication utility, there are conflicting data in the literature regarding its use as a predictive biomarker in NSCLC.[65,68] Interestingly, most studies support the notion that *KRAS* mutations are less common in Asians compared to their white counterparts.[9,69,70] In 2 studies conducted in a population of Chinese NSCLC patients, the *KRAS* mutation was found to range between 3.8% and 8%. This is dramatically low compared to the NSCLC white patient population in which studies including 2 meta-analyses suggested a prevalence rate ranging between 18% to 26%.[23,46,56,71,72] Worthy of note, *KRAS* mutational frequency among Asian never smokers was detected at an even lower range between 1.92% to 3.5%.[31-33] Yet, as with *EGFR*, there is scarce and inconsistent evidence regarding the *KRAS* mutational status in the African American group, raising the issue of health disparities in research. Some studies suggest that there is no significant difference between African Americans and whites in *KRAS* mutation frequency.[46,73] In a recent study, *KRAS* mutations were found to be more likely in whites, with 26% versus 17% in African Americans.[56] The *KRAS* mutational frequency rate seems to be quite similar in the Latin American population, ranging between 14.6% to 16.6%.[57,58] It is noteworthy that *KRAS* mutations tend to be less common in never smokers compared to former or current smokers.[69]

As with *EGFR*, the *KRAS* mutational status seems to also vary between the primary tumor and the corresponding metastases. Several studies observed the absence of *KRAS* mutations from metastases at a discordance rate of 22.5% to 26% in *KRAS*-positive lung cancer patients.[60-62] Currently, no effective targeted therapeutics targeting mutated *KRAS* exists for clinical use, although a number of agents such as

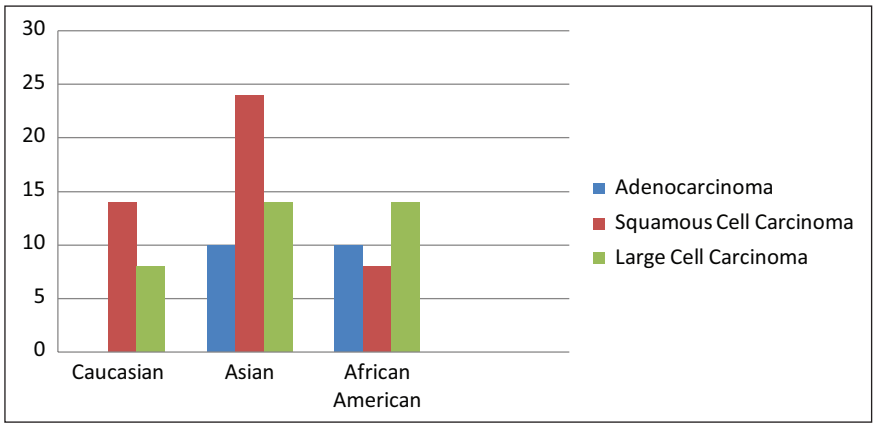

Figure 5. Spectrum of *MET* mutations among different racial groups with NSCLC.[89] The frequency of *MET* mutations is presented in accordance with the findings of the histological subtypes of lung cancer and racial groups.

MEK inhibitors are actively undergoing clinical trial investigation.

MET (c-Met proto-oncogene, HGF receptor): oncogenic mutations, amplification/overexpression, and alternative splicing. The *MET* proto-oncogene is located on human chromosome 7, where *EGFR* and hepatocyte growth factor (*HGF*) genes also reside (Fig. 5). *MET* encodes a receptor tyrosine kinase (RTK), which binds to its natural ligand, HGF, also called scatter factor (SF).[74] The ligand-receptor binding induces a conformational change in the MET receptor that facilitates receptor phosphorylation and activation. In the context of malignancy, the MET-HGF/SF pathway has been strongly implicated as a mediator of pleiotropic effects such as tumor growth, survival, branching morphogenesis, motility and migration, cell scattering, invasion, tumor angiogenesis, and metastasis.[75-77] According to a recent study by Rikova *et al*.,[78] the MET receptor ranked first in the number of tyrosinated phosphopeptides in NSCLC tissue samples among members of the RTK family, thus supporting the notion that MET is a key driver RTK in the lung oncogenic process. Furthermore, cross-talk was discovered between MET and EGFR signaling pathways in lung cancer.[79,80]

Oncogenic mutations in *MET* were first identified by Schmidt *et al*.[81-83] in 1997 in which germline mutations were found to occur in 100% of hereditary papillary renal cell carcinomas and somatic mutations in *MET* were found in 10% to 15% of sporadic papillary renal cell carcinomas. More recently, *MET* gene mutational research efforts revealed various *MET* mutations in both SCLC and NSCLC, particularly in the nonkinase domain regions. These mutations were clustered in 2 main hot spots: the extracellular ligand-binding semaphorin (Sema) domain and the regulatory juxtamembrane (JM) domain.[84-87] Interestingly, the JM mutations of *MET*[84] and also the alternative splicing variants that skip the JM domain[87,88] have been found to be oncogenic and could result in enhanced tyrosine phosphorylation, which in turn activate multiple downstream pathways that increase cell motility and metastasis.[88]

NSCLC commonly overexpresses MET, often in its activated form, and there can be frequent co-expression with EGFR.[85] In fact, MET overexpression has been found to inversely correlate with survival in lung cancer, among other human malignancies.[85] Although the MET receptor has been extensively studied, there is insufficient literature regarding its mutational profile across different racial groups. In a recent study by Krishnaswamy *et al*.,[89] lung cancer tissue samples were obtained from patients of different racial backgrounds for mutational sequencing. Notably, in this study, most of the *MET* mutations were found to be of germline nature. Interestingly, preclinical studies in germline *met* mutations in a murine transgenic model reveal mutation- and background-associated differences in tumor profiles.[90] The most frequently detected *MET* mutation, N375S, occurred in 13% of East Asians compared to only 2.6% in whites and none among African Americans. *MET* mutations were also more likely to exist in male smokers and squamous cell carcinoma. This study shows that *MET* displays different mutational profiles across different racial groups, and thus, it would be very interesting to expand on these findings and investigate the *MET* gene alterations across larger populations. The Sema domain housed all the nonsynonymous mutations except R988C and T1010I in the JM domain. The previously mentioned N375S mutation, which existed at a higher frequency in East Asians, was a nonsynonymous mutation in the Sema domain. On the contrary, R988C was only identified in whites and African Americans. This study did not detect any nonsynonymous mutations in the tyrosine kinase domain; however, whites and East Asians were found to possess a synonymous SNP, 3912C>T (D1304), in the tyrosine kinase domain more frequently than African Americans. Another earlier study identified that the somatic exon 14–skipping splice variant of *MET* occurred in 1.3% of a US lung cancer cohort.[85] This finding was echoed by similar results of 1.7% in another study focused on a Japanese cohort.[91] The Japanese study was also able to identify an increased *MET* copy number in 5.6% of the patients ($P = 0.041$).

To this end, the potential impact of *MET* alterations in tumors in the therapeutic sensitivity profile is still relatively

uncertain. A large number of MET and HGF targeting agents have been undergoing clinical trial studies in recent years, and 2 of them, tivantinib (ARQ197, a highly selective non–ATP-competitive TKI) and onartuzumab (MetMAb, a 1-arm monoclonal antibody), have already entered into phase 3 randomized clinical studies.[92,93] The phase 3 nonsquamous NSCLC study for tivantinib (ARQ197) has been completed and interim analysis showed no statistically significant difference in overall survival benefit between tivantinb plus erlotinib versus erlotinib plus placebo. Nonetheless, there is a progression-free survival benefit of the tivantinib arm over placebo arm. *MET* amplification[94] and HGF autocrine signaling[95] have been found to predict MET inhibition sensitivity in human cancers. On the other hand, *MET* mutations can pose varying degrees of impact on MET TKI sensitivity/resistance profiles in preclinical studies.[18,96] Clearly, further investigations into the role of *MET* alterations in predicting therapeutic sensitivities are urgently warranted.[97] Nonetheless, this task could prove to be more daunting than expected due to at least the versatility of the receptor oncogenic signaling cascade, which can mediate "oncogenic dependence," which promotes oncogene-addicted tumor cell proliferation and survival, and "oncogenic expedience," which promotes tumor cell invasion and metastasis. Furthermore, many of the MET targeting agents under clinical investigations are multitargeted agents, for example, cabozantinib (XL184 targeting MET, VEGFR2, RET, and AXL), with perhaps the exception of tivantinib and onartuzumab.

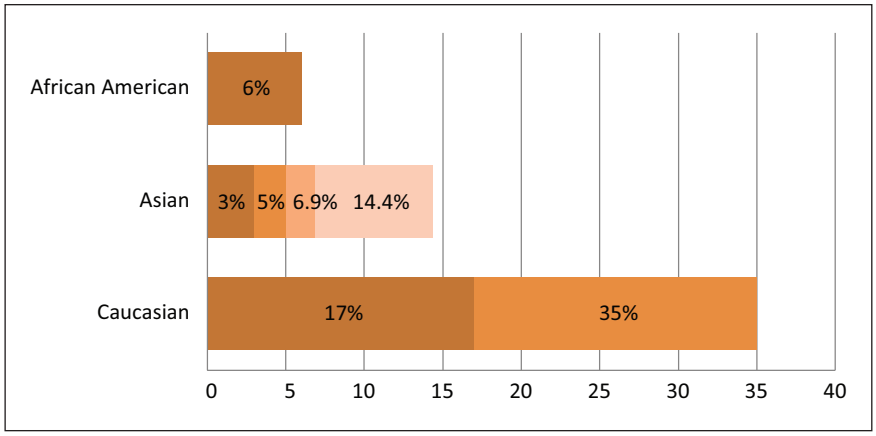

Figure 6. Spectrum of *LKB1* oncogenic mutations among different racial groups with NSCLC.[100-102,104] The different color shades represent *LKB1* mutational rates reported by different studies.

LKB1 (STK11). *LKB1* gene (also known as *STK11*) is a tumor suppressor gene located on chromosome 19p13.3 (Fig. 6). The *LKB1* gene was initially identified as the causative agent behind Peutz-Jeghers syndrome through a germline inactivating mutation.

LKB1 mutation is typically rare in most types of cancer, with the exception of pancreatic cancer, where it is present in 4% of cell lines and primary tumors and also NSCLC. It was discovered that *LKB1* possesses inactivating mutations in NSCLC tumors and was found to be a fairly common event.[98] Interestingly, mutations in the *LKB1* tumor suppressor gene were found to widely vary in frequency across different racial groups. *LKB1* mutational frequency as identified in multiple studies was reported in approximately 17% to 35% of NSCLC in the white population compared to only 3% to 7% in the Asian NSCLC population.[99-101]

Early studies on NSCLC primary tumors and cell lines of undetermined racial background reported an average *LKB1* mutation rate of 33% to 39%.[98,99] A study by Koivunen *et al*.[100] observed the *LKB1* mutation to be higher in NSCLC tumors in the US population (17%) compared with 5% of NSCLC in the Korean population. In another study conducted on a sample of Chinese lung adenocarcinomas, *LKB1* mutational frequency was found to be similar at 6.9%, while another study conducted in Japan observed a rate of 3%.[101,102] Another interesting finding reported in the literature was the F354L mutation that occurred in 6.1% of Korean lung cancer patients.[103] Similarly, a study conducted by Suzuki *et al*.[104] in Chiba, Japan, observed 14.4% *LKB1* mutations in lung adenocarcinoma samples, all of which expressed F354L substitutions. In another recent study, Gill *et al*.[105] reported that the occurrence of a homozygous deletion of *LKB1* was significantly more frequent in whites (35%) than in African American patients (6%).

Unlike *EGFR*, co-existing *KRAS* activating mutations were found with *LKB1* inactivating mutations in lung cancer samples.[98] Mutations associated with smoking history and *KRAS* mutations were found to be almost mutually exclusive with *EGFR* mutations.[100] The *LKB1* mutations also tended to occur more commonly in adenocarcinomas than in squamous cell carcinomas, where the inactivation of *LKB1* was observed at a rate of 34% and 19%, respectively, but remains a common event in both histological subtypes.[106]

BRAF. BRAF kinase belongs to a family of serine-threonine protein kinases that includes ARAF, BRAF, and CRAF (RAF1). Mutant *BRAF* has been implicated in the pathogenesis of several cancers, including melanoma, NSCLC, ovarian cancer, papillary thyroid cancer, and colorectal cancer. The most commonly identified *BRAF* mutation is V600E, which accounts for 90% of *BRAF* mutations in melanoma. In NSCLC, *BRAF* gene mutations were identified in 1% to 3% of all samples.[12,107-109] In a recent study that sampled 697 patients with lung adenocarcinoma, 18 patients tested positive for *BRAF* mutations, all of whom

were white.[110] The identified *BRAF* mutations were V600E (50%), G469A (39%), and D594G (11%). It is also noteworthy that no patient with a *BRAF* mutation had a concomitant mutation in *EGFR* or *KRAS* or a translocation in *ALK*. Most recently, a mutated *BRAF*-specific inhibitor, vemurafenib, has been approved for clinical use in V600E *BRAF*-mediated cutaneous melanoma. This raises the possibility of matching the BRAF inhibitor to mutated *BRAF* expressing NSCLC in the future and would be worth investigating.

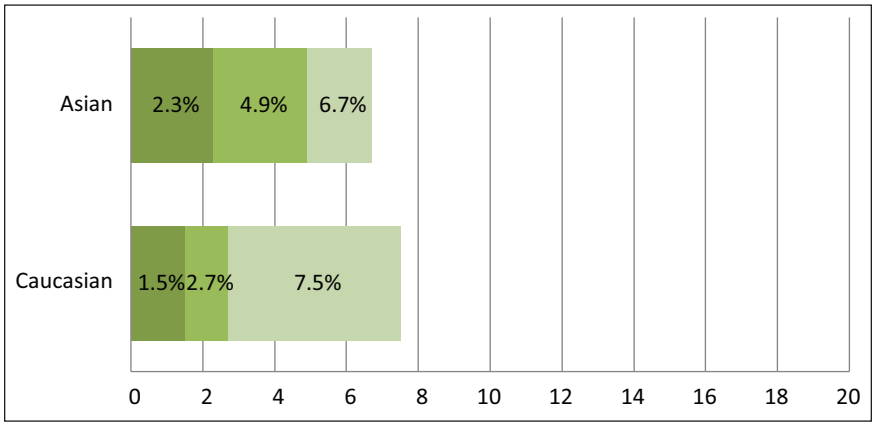

Figure 7. Spectrum of *EML4-ALK* oncogenic driver fusions among different racial groups with NSCLC.[114,116,119,120,122,124,146] The different color shades represent *EML4-ALK* rates reported by different studies. Data among human populations other than white and Asian are lacking thus far.

PIK3CA. The *PIK3CA* gene encodes p110α, one of the catalytic subunits of phosphatidylinositol 3-kinases (PI3Ks), which belongs to a family of lipid kinases involved in many cellular processes, including cell growth, proliferation, differentiation, motility, and survival. PI3K is a heterodimer composed of 2 subunits: an 85-kDa regulatory subunit (p85) and a 110-kDa catalytic subunit. PI3K converts PI(4,5)P2 to PI(3,4,5)P3 on the inner leaflet of the cell membrane. PI(3,4,5)P3 recruits important downstream signaling proteins, such as AKT, to the cell membrane, resulting in increased activity of these proteins. *PIK3CA* was found to be mutated in over 30% of colorectal cancers.[111] Somatic mutations in *PIK3CA* have been also found in 1% to 3% of all NSCLCs.[111,112] Most of the mutations tended to cluster within 2 mutational hot spots. They also tended to occur more commonly in squamous cell carcinoma.[112] *PIK3CA* shows significant potential as a candidate in cancer-targeted drug therapy. Currently, there are several ongoing clinical trials using PI3K inhibitors. Of interest, the PI3K inhibitor may also have a role to overcome acquired EGFR TKI–resistant disease since *PIK3CA* mutations have been identified in these tumor tissues in a rebiopsy study.[52]

Oncogenic Chromosomal Gene Rearrangements

ALK: Oncogenic Chromosomal Translocations. ALK is another tyrosine kinase receptor that is abnormal in various types of malignancies (Fig. 7). While the role of *ALK* in human cancer has long been recognized in *NPM-ALK* fusion in non-Hodgkin lymphoma,[113] *EML4-ALK* fusion was documented in the literature for the first time in NSCLC only recently by Soda *et al*.[114] in 2007 as a novel potential oncogenic driver mutant kinase.[115] Approximately 3% to 7% of lung tumors harbor *ALK* fusions.[114-116] Multiple different *EML4-ALK* fusion variants have been described in NSCLC, typically with varying fusion sites at *EML4* but with a constant fusion site within *ALK*.[78,114,115,117-119] *EML4-ALK* fusions are usually found in light (<10 pack years) or never smokers who tend to be of a younger age.[116,119-121] The *EML4-ALK* oncogenic rearrangement was also found to be different across different racial groups. In the Asian cohort, several studies determined the incidence of the oncogenic translocation to be in the range of 2.3% to 6.7% with no significant difference compared to Asian never smokers.[31-33] On the other hand, the rate of the *EML4-ALK* rearrangement was found to be much lower in whites, with most studies supporting a range between 1% to 3%.[114,119,120,122,123] Interestingly, according to one study conducted on a cohort of NSCLC specimens collected from Italy and Spain, the incidence rate was found to be more similar to the Asian cohort at 7.5%.[124]

In most cases, *EML4-ALK* fusions were nonoverlapping with other oncogenic mutations of *EGFR* or *KRAS*.[119-122] The presence of *EML4-ALK* fusions is also associated with EGFR TKI resistance.[119,121,122] Although the relationship between *EML4-ALK* and *MET* is not well established yet, crizotinib, a drug initially developed as a MET inhibitor, has recently been approved by the US Food and Drug Administration (FDA) for the treatment of *EML4-ALK*–positive NSCLC and can now be prescribed as a first-line treatment.[125] In a recent study by Shaw *et al*.,[126] *ALK*-positive NSCLC patients on crizotinib therapy were associated with improved survival compared to crizotinib-naive controls. Unfortunately, as with other targeted cancer drugs, patients eventually develop a resistance against crizotinib. Novel resistance mutations are continually identified. Choi *et al*.[127] reported 4374G→A and 4493C→A in the *ALK* gene, followed by Sasaki *et al*.,[128] who reported the F1174L mutation in the *ALK* kinase domain. With NGS platform analysis, more novel forms of *ALK* fusion have recently been uncovered, such as *C2orf44-ALK* in colorectal cancer.[129] Further fusion variants of human oncogenic translocations in lung cancer would be expected to be generated

with more efficient and affordable NGS efforts as applied in different human populations in the near future.

RET. The *RET* proto-oncogene encodes a RTK for the glial cell line–derived neurotropic factor family of ligands; RET signaling is essential for neural development and maintenance.[130] *RET* mutations are known to be present in thyroid cancers. In fact, 10% to 20% of all sporadic papillary thyroid carcinomas contain *RET* fusions in which 60% to 70% of them are attributed to *RET-PTC1* fusion.[131,132] In lung cancer, *KIF5B-RET* fusion was recently discovered in a small cohort of NSCLC patients, with 0.8% (1/121) of the patients who tested positive being of European ancestry and 2% (9/405) of Asian descent. Interestingly, there were no known oncogenic mutations detected in *RET*-positive patients, suggesting that *RET* fusion might be the driving force behind the oncogenic process. In another study by Kohno *et al*.,[133] they reported the presence of *KIF5B-RET* fusion in 1% to 2% of lung adenocarcinomas in both Asians and non-Asians. The relation between smoking and *KIF5B-RET* fusion remains unclear. Worthy of note, *PTC-RET*–positive thyroid cancers are sensitive to sorafenib, a RET inhibitor; this raises the notion to test RET kinase inhibitors in patients with *KIF5B-RET*–positive NSCLC to determine potential clinical benefits.[129] Moreover, RET can also be targeted by a novel kinase inhibitor cabozantinib (XL184), which also has inhibitory activities against MET, VEGFR2, and AXL besides RET and will be formally tested in an upcoming phase 2 NSCLC study with *RET* fusion patients.

ROS1. *ROS1* is a RTK of the insulin receptor family. The gene was originally found to be fused to the adjacent *FIG* gene in glioblastomas. Later on, *ROS1* fusions were identified in approximately 2% of NSCLCs and postulated as a potential driver mutation.[134,135] These fusions resulted in RTK activation, although the details of the downstream signaling transduced by *ROS1* fusion are not fully understood yet. In a recent study by Bergethon *et al*.,[136] they reported that patients with *ROS1* rearrangements were significantly younger, more likely to be never smokers, and were overrepresented in the Asian race. Interestingly, they were also able to show that ROS-positive status was associated with sensitivity towards TKIs, specifically crizotinib, with a patient demonstrating prompt and durable complete response. Overall, *ROS1* as a potential therapeutic target in lung cancer, and its molecular alterations and racial differences and determinants of inhibitor sensitivity and resistance, would warrant further definition and investigation.

Genetic Polymorphisms in Lung Cancer: Impact on Targeted Therapy

Studies show that genetic polymorphic variations of *EGFR* have an association with *EGFR* mRNA expression.[137] Interestingly, the –216G/T polymorphic variant either alone or with –191C/A located in the *EGFR* gene promoter transcriptional start site was associated with either better clinical outcome or increased gefitinib toxicity or both.[137,138] These genetic polymorphic variations were both found in white patients and were relatively rare in Asians.[139] Other studies investigating the intron 1 enhancer element among different racial groups tried to correlate the CA repeat length to outcome, EGFR expression, and gene copy number along with EGFR TKI therapeutic efficacy and toxicity.[140] Unfortunately, these gene polymorphism studies were found to be difficult to interpret and not very reliable because they were underpowered with multiple confounding factors.

Lung Cancer Genome Analysis: NGS and Future Directions

In this review, we highlighted several recent examples of novel lung cancer genome alterations such as *KIF5B-RET* fusions[129,133] that were uncovered using high-throughput NGS of tumor samples. In a study by Chmielecki *et al*.,[141] they were able to map out tyrosine kinase fusions in the conserved GXGXXG kinase motif in a NGS study. In another recent study by Pleasance *et al*.,[142] investigators identified 22,910 somatic substitutions in a single SCLC cell line, "NCI-H209," in whole genome sequencing analysis. These mutations were found to represent the effects of carcinogens associated with tobacco smoking. NGS and high-throughput cancer genome decoding in recent years have undeniably brought forth a genomic revolution in cancer research and clinical personalized cancer therapeutics. The new generation of non-Sanger–based sequencing technologies has delivered on its promise of sequencing DNA at unprecedented speeds, thereby enabling impressive scientific achievements and novel biological application. As the cost of NGS declines at a rapid pace, and more novel and faster NGS platforms continue to emerge in the market, the process of cancer genome sequencing is already undergoing a rapid "democratization process." NGS of cancer genomes is becoming more readily available beyond just a handful of large academic or industrial genome centers. Global genome analysis is also underway and would ultimately facilitate a deeper understanding of human cancer genome variations, including lung cancer, among different human racial populations.

Although all these advancements are very exciting, many challenges still lie ahead. First, the enormous bioinformatics output will require an extensive amount of talent and expertise that is likely the "bottleneck" for further progress in genome science. Second, the digital and global sharing of patient genomic information poses many ethical, legal, and socioeconomic issues and concerns that transcend territories of patient privacy and health care insurability. Nonetheless, there are accelerated efforts from the government (National Institutes of Health/National Human

Genome Research Institute [NIH/NHGRI]) to summon resources to research the legal and social impact of clinical cancer genome sequencing to keep pace with the technological advances but also to facilitate their translational leap into clinical patient care benefits. On the other hand, as the technological advances bring forth more sophisticated than ever sequencing platforms and capacity, it cannot be overemphasized that the source and quality of the tumors to be sequenced still hold the key to the validity of the resultant data and integrity of the published literature ultimately. Detailed and accurate annotation of the source of tumor DNA, as well as processing methodology, should be presented in such studies. For instance, the use of laser microdissection or whole genome amplification and the specific sequencing method should be reported. Moreover, it would be crucially important to specify in studies whether the sequencing was performed from primary versus metastatic tumor sites[59-62] and, if the latter, which specific organ site of metastasis.

Finally, despite all the challenges, it is perhaps now not unrealistic to anticipate that implementing tumor genomic profiling using NGS across different human populations in lung cancer would enormously expand our knowledge base in lung cancer biology and racial disparities in the disease outcome. It is also expected that a more wide adoption of clinical cancer genome sequencing would open new fronts into the development of molecularly personalized cancer therapy.

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