Genetic Changes in Squamous Cell Lung Cancer: A Review

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Abstract

Identifying specific somatic mutations that drive tumor growth has transformed the treatment of lung cancer. For example, cancers with sensitizing epidermal growth factor receptor mutations and echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase translocations can have remarkable responses to epidermal growth factor receptor and ALK inhibitors respectively, leading to significant clinical benefit. However, effective molecularly targeted therapies have disproportionately impacted adenocarcinomas compared to squamous cell carcinomas, and never or light smokers compared to heavy smokers. Further progress in non–small-cell lung cancer will require the identification and effective targeting of molecular alterations in all subtypes of lung cancer. Here, we review the current knowledge about the molecular alterations found in squamous cell carcinoma of the lung. First, we will discuss the ongoing efforts to comprehensively assess the squamous cell carcinoma genome. We will then discuss the evidence supporting the role of specific genes in driving squamous cell carcinomas. By describing the landscape of somatic targets in squamous cell lung cancer, we hope to crystallize the current understanding of potential targets, spur development of therapies that can have clinical impact, and underscore the importance of new discoveries in this field.

Keywords

Squamous cell carcinoma of the lung; Molecular targets; Somatic mutations

SQUAMOUS CELL CANCER IS A DISTINCT THERAPEUTIC SUBSET

Lung cancer is the leading cause of cancer-related deaths in the United States, with more than 220,000 new cases and more than 157,000 deaths annually.1 Approximately 85% of the newly diagnosed lung cancers are non–small-cell lung cancer (NSCLC), and of these, approximately 30% are squamous cell carcinoma. Historically, approaches to the treatment of NSCLC were uniform, and histologic subtypes within NSCLC did not significantly affect treatment decisions.2 However, recent advances in NSCLC drug development have introduced histology as an important factor that can alter treatment options. For example, bevacizumab is contraindicated in patients with squamous cell lung cancer, because of an increased risk of fatal hemoptysis.3,4 Pemetrexed is approved for the treatment of non-squamous NSCLC only, as multiple studies have shown greater efficacy in nonsquamous as compared to squamous cancers.5-8
Although the selection of drugs by histology is one refinement on the previous approach, ultimately the field is evolving toward classifying lung cancers according to the specific somatic mutations that drive tumor growth. For example, cancers with sensitizing epidermal growth factor receptor (EGFR) mutations and echinoderm microtubule-associated protein-like 4 (EML4)-anaplastic lymphoma kinase (ALK) translocations can have remarkable responses to EGFR and ALK inhibitors respectively, leading to significant clinical benefit. However, effective molecularly targeted therapies have disproportionately impacted adenocarcinomas compared to squamous cell carcinomas, and never or light smokers compared to heavy smokers. Further progress in NSCLC will require the identification and effective targeting of molecular alterations in all subtypes of lung cancer.

Here we review the current knowledge about the molecular alterations found in squamous cell carcinoma of the lung. First, we will discuss the ongoing efforts to comprehensively assess the squamous cell carcinoma genome. We will then discuss the evidence supporting the role of specific genes in driving squamous cell carcinomas. By describing the landscape of somatic targets in squamous cell lung cancer, we hope to crystallize the current understanding of potential targets, spur development of therapies that can have clinical impact, and underscore the importance of new discoveries in this field.

COMPREHENSIVE GENOMIC STUDIES IDENTIFYING SOMATIC GENE ALTERATIONS IN LUNG SQUAMOUS CELL CANCER

Mutations

Next-generation sequencing technologies are allowing better characterization of cancer genomes. Multiple investigators have reported genomic alterations in cancers, using high-throughput sequencing technologies. In lung cancer, efforts assessing somatic mutation and copy number alteration (CNA) profiles were initially confined to adenocarcinoma. More recently, studies have included squamous cell lung cancers as well. Kan et al. identified 2500 somatic mutation events in 967 of the 1507 candidate genes studied across a broad range of tumor types. In general, lung cancers had a high rate of protein-altering mutations, with adenocarcinomas and squamous cell carcinomas of the lung having rates of 3.5 and 3.9 per Mb respectively, compared to the rate of 1.8 per Mb across all tumor types. This attests to the genomic complexity of lung cancers and the comparative difficulty of effectively treating these tumors. As would be expected, mutated cancer genes identified in squamous cell lung carcinoma are distinct from the cancer genes found in lung adenocarcinoma. Some of the more commonly mutated genes observed in squamous cell lung cancers included TP53, GRM8, BAI3, ERBB4, RUNX1T1, KEAP1, FBXW7, KRAS, among others. The Cancer Genome Atlas, a project funded by National Institutes of Health and National Human Genome Research Institute to define the somatic genomic changes in more than 20 different types of cancer, is actively performing analyses on lung squamous cancers, and results from this effort are expected later this year.

As systemic mutation screens are performed, it is important to separate “driver” mutations, which confer growth advantage and are causally related to cancer development, versus “passenger” mutations, which are biologically neutral and do not confer growth advantage. Although it is clear that the somatic mutation rate in squamous cell lung cancer is high, indeed higher than what is seen in many other solid cancers, it is possible that many of the observed mutations may be “passenger” mutations. Identifying strategies to effectively match drug therapies to tumors relies on targeting the key “driver” mutations, so this distinction will be critical.

One potential method to help identify driver mutations may be to interrogate signaling pathways to see which genes are actually activated in these cancers (Fig. 1). Rikova et al. 
investigated phosphotyrosine signaling in lung cancer cell lines and tumors to identify aberrant tyrosine kinase signaling in lung cancer. Squamous cell cancers that were tested in this way showed activation in multiple kinase pathways, including DDR1, DDR2, VEGFR1, VEGFR2, PDGFRα, MET, EPHA2, and EPHB3. As discussed later, these genes are among the key candidate driver mutations thought to be altered in squamous cell lung cancer.

Copy Number Alterations

In addition to mutations, comparative genomic hybridization studies and loss of heterozygosity studies have demonstrated the frequency with which CNAs can occur in cancer. CNAs can help identify areas of amplification or loss of genetic material, which may signal oncogenic or tumor suppressor candidate genes. Recurring CNAs in lung cancer have been identified and most frequently involve gains in 1q31, 3q25–27, 5p13–14, 8q23–24, and losses in 3p21, 8p22, 9p21–22, 13q22, 17p12–13, among others. Although many of these areas of CNA are common to both squamous carcinoma and adenocarcinoma of the lung, a few regions seem to be more common in squamous histology, including gain in 3q26 and gain in 8p12. Candidate genes in these areas include PI3KCA, SOX2, p63, SSCRO/DCUND1, TERC, in 3q26, and BRF2, FGFR1, and WHSC1L1 for 8p12.

CANDIDATE GENES IN SQUAMOUS CELL LUNG CANCER

In this part of the review, we will discuss specific candidate genes and the literature supporting their roles as oncogenic drivers in squamous cell lung cancer. A literature search was conducted for articles on PubMed published between 1990 and 2011, using the search terms “squamous cell lung cancer,” which were combined with the terms “somatic mutations,” “amplification,” “genetics,” and “loss of heterozygosity.” Articles not available in English were excluded. Germline genetic changes and microRNA alterations were excluded as they are beyond the scope of this review. Further specific searches were performed in PubMed for the individual genes that were reported in genomic studies that included squamous lung cancer. In this review we focused primarily on activating mutations and overexpression or amplification as the genomic change of interest rather than loss of expression, because targeted therapies have to date been most successful against these classes of genetic changes. However, it is possible though not yet proven that some tumor suppressor changes, such as PTEN loss, may be successfully targeted, for example with phosphatidylinositol 3-kinase (PI3K) inhibitors; therefore, we have included some discussion of this topic. Overall, the primary focus of this review is on two classes of gene alterations: (1) overexpression or amplification and (2) point mutation. Notably, some candidate genes demonstrate both mechanisms of activation (Table 1). Both types of genetic activation can potentially be effectively targeted with therapeutic agents. For example, trastuzumab is effective in HER2-amplified breast cancers and gefitinib or erlotinib is effective in EGFR-mutant lung cancers. Which of the alterations reported in squamous cell lung cancer are truly “driver” mutations, and could be effectively targeted, remains to be seen and is an area of active inquiry. Table 2 shows some of the drugs currently in clinical trials, which target specific oncogenic changes and may be relevant in squamous cell lung cancer.

SOX2

Amplification of 3q26 is one of the most frequently reported alterations in squamous cell lung cancer. SOX2 has been implicated as a candidate gene in this area, with high-level amplification of SOX2 reported in approximately 20% of lung squamous cell carcinomas. SOX2 is a transcription factor and a critical regulator of normal stem cell...
function in embryonic and neural stem cells; it is thought to play a key role in the development of lung epithelium. Bass et al. showed that RNA interference knockdown of SOX2 reduced cellular proliferation, and that suppression of SOX2 had a greater effect on growth compared to other candidate genes at the 3q26 locus including PIK3CA and TP63. However, SOX2 alone was not transforming, which is generally a requirement for a “driver” event. Similarly, Hussenet et al.\textsuperscript{41} showed that overexpression of SOX2 in cell culture leads to migration and anchorage independent growth, and knockdown of SOX2 impairs growth. However, difference in cell-invasion capacity was not seen, and the rate of tumor growth was slow, suggesting that other hits are required to be fully transforming.\textsuperscript{41} Overexpression of SOX2 in mouse models leads to extensive hyperplasia and carcinoma in those with the highest levels of SOX2.\textsuperscript{42} The bulk of evidence suggests that SOX2 amplification may represent a “priming event” that requires additional downstream events\textsuperscript{43} to be fully transforming. There are no SOX2 inhibitors in clinical trials at the moment (see Table 2 for specific targeted agents in clinical trials).

**PIK3CA**

PIK3CA is another candidate gene that is found in the 3q26 amplified area. PIK3CA encodes the gene for the catalytic subunit (p110\textsubscript{α}) of PI3K. The PI3K-AKT pathway plays a central role in the survival and proliferation of many cancers.\textsuperscript{44} Both copy-number gains and mutations in PIK3CA have been identified in lung cancer. PIK3CA copy-number gains occur in approximately 20% of lung cancers, with higher frequency in squamous cell carcinomas.\textsuperscript{45–47} Amplification presumably leads to activation of PI3K pathway cell signaling, but the details of this mechanism are not yet clear.

Somatic mutations in PIK3CA have also been described and promote activation of the PI3K-signaling pathway.\textsuperscript{48} Mutations in PIK3CA are clustered in two hotspot regions in exons 9 and 20 encoding the helical and kinase domains of the protein, respectively. These mutations lead to increased lipid kinase activity and constitutive PI3K-AKT signaling.\textsuperscript{48,49} The mechanism of action is different based on mutation type; for example, the helical-domain mutants E545K and E542K interfere with the inhibitory interaction between the regulatory subunit p85 and the catalytic unit p110\textsubscript{α}, whereas the kinase domain mutant H1047R is located near the activation loop, and leads to constitutive signaling through the kinase.\textsuperscript{49} PIK3CA mutations have been reported in 1% to 5% of the NSCLC cell lines and tumors.\textsuperscript{45,50} Kawano et al.\textsuperscript{50} found PIK3CA mutations in 6.5% of lung squamous cell carcinomas, and less often in lung adenocarcinomas (1.5%). There are multiple PI3K inhibitors in development, with specificity ranging from dual PI3K/MTOR inhibition to pan-PI3K and isoform-selective PI3K inhibitors (see Table 2). Preclinical data suggest that cancers harboring activating mutations in PIK3CA are among the most sensitive to single-agent PI3K-pathway inhibitors.\textsuperscript{49} As clinical trials with PI3K-pathway inhibitors accrue squamous cell carcinomas with PIK3CA mutations, we need to examine if these cancers are truly addicted to PI3K signaling. Combinations of PI3K inhibitors with inhibitors of other cancer-related pathways are also being actively tested as investigators try to suppress multiple therapeutic pathways.

**FGFR1**

Amplification at 8p12 was observed in multiple studies of squamous cell lung cancer,\textsuperscript{39,51} and FGFR1 has been identified as a potential candidate gene in this region. FGFR1 is a member of the FGFR family of receptor tyrosine kinases; activation leads to downstream signaling via PI3K/AKT, RAS/MAPK pathways that are central to growth, survival migration, and angiogenesis in many cancers.\textsuperscript{52} Dysregulation of the FGFR1-4 signaling has been described in multiple cancers, with overexpression seen in breast, prostate, myeloma, and point mutations observed in sarcoma, bladder, and endometrial cancers, among
In lung cancer, FGFR1 amplification was enriched in squamous cell cancers in comparison to adenocarcinomas, with approximately 20% of squamous cells having FGFR1 amplification. Inhibition of FGFR1 both in cell lines and in mouse models with FGFR1-amplified engrafted tumors showed growth inhibition and induced apoptosis. FGFR1 mutations were rare. Multiple FGFR inhibitors are in development; many of these are multitargeted tyrosine kinase inhibitors (TKIs), which have activity against other targets in addition to FGFR1. Some current trials are specifically targeting lung cancers harboring FGFR1 amplification (ClinicalTrials.gov).

**IGF1R**

The insulin-like growth factor pathway is important in embryonic development, growth, and metabolism, and dysregulation of the insulin-like growth factor pathway has been described in multiple tumor types. IGF1R activation triggers downstream pathways, including the RAS/RAF/MAPK pathway and the PI3K pathway, leading to cell proliferation and inhibition of programmed cell death. In lung cancer, over-expression of IGF1R (located on 15q26) is more commonly seen in squamous cell as compared to other histologies. However, genetic activation of this pathway has not been reported. A great deal of clinical interest was spurred by the early results from a randomized Phase II study of chemotherapy with or without figitumumab, an antibody against IGF1R, which showed promising activity in Phase-II studies, especially among patients with squamous cell lung cancer. Biomarker analyses in a subset of patients suggested benefit among those with higher levels of IGF1 or higher tumoral IGF1R levels in squamous cell cancers; although, numbers were small in these analyses. Oddly, higher IGF1 levels were observed in adenocarcinoma whereas those patients with squamous cell histology were reported to have more benefit in the study as a whole, throwing into question whether these biomarkers were truly predictive. Two Phase-III studies with figitumumab in combination with either chemotherapy or erlotinib were recently closed because of futility and increased toxicity, and further development of this class of agents is in question. Small molecule TKIs of IGF1R are also being studied, and a challenge with this class of drugs is hitting the IGF1R target selectively, given the crossreactivity with insulin receptor.

**EphA2**

The Eph receptor family is a group of receptor tyrosine kinases that are divided into EphA and EphB based on structural homology and ligand affinity. Eph receptors are important in embryonic development such as cell migration, vascular development, and tissue-border formation. Overexpression of EphA2 (located on chromosome 1p36) is seen in multiple cancers, including NSCLC, and is thought to promote cell motility, invasion, metastasis, and angiogenesis. EphA2 expression has been correlated with smoking and worse survival, and has been reported to be higher in metastatic lesions as compared to primary sites. In addition to overexpression, mutations in EphA2 have been described; although overall rare, they are found more commonly in squamous cell as compared to nonsquamous lung tumors. A mutation in EphA2 (G391R) was identified in two of 28 squamous cell lung cancers (7%), but not in any adenocarcinomas or large-cell lung carcinomas. Overexpression of both the wildtype and mutated EphA2 increases cellular invasiveness, with the effect being more pronounced with the mutated receptor. Overexpression of EphA2 leads to increased phosphorylation of SRC, Cortactin, and p130Cas. Of note, the activity of p130Cas is particularly important in G391R mutated cells. These findings lead to the emergence of EphA2 as a potential target for drug development. Dasatinib, a multitargeted TKI, has activity against Eph family receptors in addition to BCR-ABL, KIT, and SRC tyrosine kinases, and more specific EphA2 inhibitors are also under development.
MET

MET (located on 7q31) encodes a receptor tyrosine kinase for hepatocyte growth factor, and overexpression of MET has been linked with abnormal cell proliferation and invasion.\textsuperscript{70} Cells with MET amplification demonstrate high sensitivity to MET inhibitors,\textsuperscript{71} and a lung cancer with MET amplification demonstrated high sensitivity to the ALK/MET inhibitor, crizotinib,\textsuperscript{72} suggesting cancers with true amplification may be sensitive to MET inhibitors. MET amplification has also been identified as a mechanism of resistance for EGFR TKI therapy.\textsuperscript{73} Both copy-number gain/high polysomy and true amplification of MET have been identified in NSCLC. Although copy-number gain/high polysomy seems to have no association with specific histology, true amplification may be more common in squamous cell compared to nonsquamous.\textsuperscript{74} MET amplification was found in six of 97 squamous patients (6.2\%) and was associated with worse prognosis.\textsuperscript{74} There are multiple MET inhibitors currently being tested in clinical trials. The best selection criteria for targeting MET (i.e., overexpression versus copy-number gain versus amplification) remains to be seen; for example, a recent study showed that MetMab, an antibody against MET, had a progression-free survival and overall-survival benefit for patients who received erlotinib with MetMab versus erlotinib alone, among patients who overexpressed MET (defined as $\geq$ 50\% tumor with moderate/strong MET expression).\textsuperscript{75} Approximately 30\% of the patients on this study had squamous histology, and the numbers with squamous and MET overexpression are too small in this study to draw conclusions regarding histology. Median progression-free survival was 2.9 months for those receiving MetMab with erlotinib versus 1.5 months for erlotinib alone among those with MET overexpression ($p = 0.04$), while median overall survival was 12.6 versus 3.8 months ($p = 0.002$). On the basis of these results a Phase-III study is planned. Multiple other MET inhibitors are in clinical development.

PDGFRA/4q12 Amplification

Amplification of 4q12 has been reported in 3\% to 7\% of lung adenocarcinomas and 8\% to 10\% of lung squamous cell carcinomas.\textsuperscript{76} PDGFRA and KIT map to the region of focal amplification. Abnormalities in PDGFR have been identified in multiple cancers including hematologic malignancies, GIST, medulloblastomas, and gliomas.\textsuperscript{77} PDGFRA amplification is seen in a lung squamous cell cancer cell line (NCI-H1703), and short hairpin RNA knockdown and small-molecule inhibition of PDGFRA inhibit cell survival and anchorage independent growth, suggesting that in a subset of NSCLC PDGFRA may be an essential oncogene.\textsuperscript{76} Multiple PDGFRA inhibitors are in clinical development. Multitargeted kinases such as sunitinib, which target PDGFRA and multiple other targets have been tested in lung cancer previously, although not specifically by genotype or squamous histology; more selective inhibitors are also in development.

p53/MDM2

The p53 tumor suppressor gene (located on 17p13) functions mainly as a transcription factor, binding specific DNA sequences, and activating or repressing genes that regulate cell cycle arrest, apoptosis, and DNA repair.\textsuperscript{78} Inactivation of p53 is important for cancer cell survival across multiple tumor types, and is one of the most commonly found alterations in cancer.

Mutations in p53 are a frequent event in lung cancer, seen in more than half of NSCLCs, and approximately 65\% of squamous cell carcinomas.\textsuperscript{79} Mutational hotspots are concentrated in the sequence-specific DNA-binding domain, and approximately 75\% of mutations are missense\textsuperscript{80} and lead to loss of function as a transcription factor. The mutational spectra are affected by smoking,\textsuperscript{80,81} and show excess of G$\rightarrow$T tran-versions, which are linked to polycyclic aromatic hydrocarbon (PAH) adducts. Interestingly, there is a correlation between p53 mutational hotspots and hotspots of adduct formation by PAHs.\textsuperscript{82,83}
Accumulation of nonfunctional mutant p53 leads to high concentrations of mutant p53 in tumor cells.

In addition to mutations in p53, inactivation of wild-type p53 can be seen, which can also dysregulate the p53 pathway and promote carcinogenesis. In a substantial number of tumors, wild-type p53 is inactivated by MDM2 overexpression or amplification; normally, MDM2 and p53 are tightly regulated in a negative feedback loop where MDM2 ubiquinates p53 and marks it for degradation; overexpression of MDM2 therefore leads to inactivation of p53. MDM2 amplification (located on 12q14) has been reported in 6% to 7% of NSCLC, in both adenocarcinoma and squamous cell carcinoma and tends to be an exclusive event of p53 mutation.

Targeting the p53 axis has been difficult, as p53 is a transcription factor with complex protein–protein interactions, without an easily accessible receptor–ligand interaction or enzymatic active site that would render it a more easily druggable target. Multiple approaches to targeting p53 have been attempted, including adenovirus-based gene therapy, and more recently small molecules designed to attempt to activate endogenous p53 in tumors retaining the wild-type gene. One potential strategy is the development of small molecules that to try to increase p53 activity by neutralizing MDM2, including nutlins that bind and dissociate MDM2 from p53. Small molecules targeting mutant p53 are also in development but are an even greater challenge to develop, given the wide range of mutant proteins that are expressed.

**AKT**

A somatic mutation in AKT (located on 14q32), E17K, constitutively activates the protein kinase. The AKT1 E17K mutation was found in two of 36 squamous cell lung cancers (5.5%), but not in lung adenocarcinoma (zero of 53). Both patients with the mutation were male smokers with squamous cell carcinoma. Examples of AKT inhibitors in clinical development are shown in Table 3. It remains unknown whether cancers with these mutations will be sensitive to single-agent AKT inhibitors.

**EGFR**

The activating mutations in the EGFR (located on 7p12) tyrosine kinase domain that confer exquisite sensitivity to EGFR tyrosine kinase inhibition are found in adenocarcinoma and bronchioalveolar histologies rather than squamous cell carcinoma. However, alterations involving EGFR are found in squamous cell lung cancer in the form of copy-number gains and variant-III mutations.

In squamous cell lung cancers, high EGFR gene copy-number and protein overexpression are observed more frequently than in adenocarcinoma (82% versus 44%). Although EGFR overexpression has been associated with worse prognosis in some studies, it has not been associated with response to the EGFR TKIs used clinically. A retrospective analysis of FLEX, a large Phase-III study evaluating chemotherapy with or without the anti-EGFR antibody cetuximab, suggested that EGFR overexpression may be associated with better outcomes in the cetuximab arm, but these findings have not been confirmed in other studies.

The variant-III (vIII) in-frame deletion of exons 2 to 7 in EGFR, which was initially described in glioblastoma, causes a deletion in the extracellular domain that has an activating effect on the receptor, leading to a proliferative advantage in cells expressing these truncated receptors. The EGFR vIII mutation was found to be present in three of 56 squamous cell lung cancers (5%), but in none of the 123 lung adenocarcinomas. In mouse models, expression of the EGFRvIII mutation led to the development of NSCLC, and
inhibition with an irreversible EGFR inhibitor caused tumor regression. EGFR-vIII mutations were found in eight of 252 patients with lung cancer in one study; all were male smokers, and seven had squamous cell carcinoma. It should be noted that BR.21 showed benefit of erlotinib in squamous cell cancers and adenocarcinomas. More specific molecular correlative data regarding copy number or EGFR-vIII mutation in the squamous patients are not available; however, preclinical studies would suggest that reversible EGFR inhibitors such as erlotinib or gefitinib would not have significant activity against the vIII mutation specifically because the kinase domain of vIII would have the same affinity toward gefitinib/erlotinib as the wild-type EGFR, and thus we would not observe the same therapeutic window we observe with exon 19 and 21 mutant EGFR. However, it remains possible that cancers with vIII are “addicted” to EGFR signaling and could still be potentially sensitive to therapeutic inhibition of the EGFR-signaling pathway.

**DDR2**

DDR2 (located on 1q23) is a receptor tyrosine kinase that binds collagen and has been shown to promote cell migration, proliferation, and survival. Mutations in DDR2 have been reported in lung cancer, although with varying frequency. Looking specifically at squamous cell lung cancers, DDR2 kinase gene mutations were identified in 3.8% of squamous cell lung cancers and cell lines (nine out of 277 squamous cell tumors, 3.2%). Knockdown of DDR2 by RNA interference or by dasatinib in cell lines with DDR2 mutations led to inhibition of proliferation, and ectopic expression of mutated DDR2 led to cellular transformation, although to varying degrees. Although preliminary, these results suggest that these mutations may be oncogenic, and cancers with mutations in DDR2 may be sensitive to drugs that inhibit its kinase activity.

**LKB1**

LKB1 (located on 19p13) is a serine–threonine kinase that regulates cell-cycle progression, apoptosis, and polarity. LKB mutation rates seem to be higher in whites compared to Asian populations, and seem to be correlated with smoking and kras mutations. Although inactivation of LKB1 is more commonly associated with adenocarcinoma, it has been seen in squamous cell cancers as well. Genomic alterations in LKB1 was found in 34% adenocarcinomas and 19% squamous cell carcinomas in one study of NSCLCs, with most of these being single copy mutations or deletions. Mutations in LKB1 were detected in 11% of lung cancers tested, and more commonly in adenocarcinomas (13%) versus squamous (5%). The majority of mutations were deletions or insertions, with some missense or nonsense mutations also being observed. Interestingly, the mutations were more common in a U.S. cohort compared to an Asian one; in the United States, LKB1 mutations were seen in 19% of adenocarcinomas and 13% of squamous, whereas the Asian cohort had only an 8% mutation rate in adenoc and 0% in squamous. In mouse models, LKB1 inactivation in combination with activating mutations in kras led to lung tumor growth with frequent metastasis; LKB1 inactivation led to both adenocarcinoma and squamous tumors in these mouse models. Signaling through activated SRC and focal adhesion kinase pathways may be important in increasing cell migration and invasiveness in LKB1-deficient lung cancers, and a combination of PI3K, MTOR, MEK, and SRC inhibition may be a useful treatment strategy in these cancers.

**PTEN**

The tumor-suppressor gene PTEN (located on 10q23) encodes a lipid phosphatase that negatively regulates the PI3K/AKT pathway, and loss of PTEN leads to constitutive PI3K-AKT signaling. Somatic PTEN deletions and mutations and inactivation of PTEN by epigenetic mechanisms such as methylation or microRNA silencing are seen in multiple cancers. Reduction or loss of PTEN expression has been reported in up to 70% of
NSCLC, both adenocarcinoma and squamous cell. In addition, PTEN mutations occurring in approximately 5% of lung cancers are significantly associated with squamous cell rather than adenocarcinoma histology (10.2% versus 1.7%). Cancers with PTEN loss may be more sensitive to inhibitors of the PI3K pathway, although definitive data are lacking. Clinical trials of PI3K inhibitors in cancers with PTEN loss are ongoing and should provide much needed insight into this question.

NRF2/KEAP1

Nuclear factor erythroid-2–related factor 2 (NRF2, located on 2q31) is a transcription factor that regulates the expression of cytoprotective genes that are normally induced in response to environmental and endogenous oxidative stress. Kelch-like ECH-associated protein 1 (KEAP1, located on 19p13) negatively regulates NRF2 activity by targeting it for degradation. Somatic mutations in NRF2 have been reported in human cancers, especially among those with smoking history or squamous histology. These mutations block proper NRF2-KEAP1 binding and inhibit KEAP1-mediated degradation of NRF2. NRF2 mutations have been found in 8% to 11% of lung cancers in predominantly squamous histology, and have also been reported in squamous cell cancers of esophageal, larynx, and skin primary sites. In contrast, KEAP1 mutations and loss of heterozygosity of KEAP1 have been identified in lung cancer and lead to NRF2 activation; however, these have been seen predominantly in adenocarcinoma, although the numbers analyzed to date have been small. In addition to mutations, aberrant NRF2 and KEAP2 expression is found in lung cancer at rates higher than mutation rates, suggesting that other mechanisms for NRF2/KEAP1 pathway dysregulation are involved as well: NRF2 expression was found in 26% of NSCLC, more commonly in squamous versus adenocarcinoma (38% versus 18%, p < 0.0001), whereas low or absent KEAP1 was significantly more common in adenocarcinoma than squamous (62% versus 46%, p = 0.0057). There are currently no specific drugs targeting these genetic changes, but efforts are currently underway to identify targets that may be specifically lethal in this subset of lung cancers.

Smoking-Related Cancers and Genetic Changes

Squamous cell cancers are typically found in smokers, and it is possible that the squamous cell carcinomas of the aerodigestive system (e.g., lung, esophagus, and head and neck) share common genetic alterations. For example, SOX2 amplification has been reported in both squamous cell carcinomas of the lung and the esophagus, and NRF2 mutations have been found in squamous cell cancers of the esophagus and head and neck. As more is learned about the specific genetic changes in different tumor subtypes, we may indeed find common themes that link the squamous cell cancers of different primary tumor types.

This seems highly likely, given the common risk factor of tobacco smoke underlying these cancers. Multiple carcinogens have been found in cigarette smoke, including PAHs, azaarenes, N-nitrosamines, aromatic amines, heterocyclic aromatic amines, and aldehydes. At least 50 carcinogens in cigarette smoke have been identified that cause lung tumors in animals or humans. Metabolic activation of these carcinogens leads to DNA adduct formation, whereby the active metabolite binds covalently to DNA. These DNA adducts distort the DNA helix and lead to aberrant coding, and ultimately the accumulation of mutations leads to loss of normal controls on cell growth. In addition to the overall increase in mutational frequency caused by carcinogens from tobacco smoke, there are specific hotspots of mutations seen. For example, a dose response relationship between tobacco smoke and p53 mutations has been shown, and G to T transversions in p53 are more common among smokers than among nonsmokers. Interestingly, newer technologies suggest that such changes may be more global than previously recognized. Pleasance et al. performed deep sequencing of a small-cell lung cancer cell line to

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explore the mutational burden associated with smoking. The number of somatic substitutions that were identified was 22,910, of which 134 (0.6%) were in coding exons. G->T or C->A transversions were the most common change, a pattern very similar to what is observed in p53. One important challenge will be the sheer volume of mutations found in the cancers of heavy smokers. As noted previously, the classical “driver” mutations of EGFR and ALK found in lung cancer have been found predominantly in never-to-light smokers. It is possible that the more complex genetic mutation burden in smokers will make identifying true drivers in this population more challenging.

CONCLUSIONS

The treatment of lung cancer has been revolutionized by the discovery of specific targeted therapies such as erlotinib or gefitinib for EGFR-mutated lung cancer and crizotinib for ALK-translocated lung cancer. These successes have taught us that lung cancer is not monolithic, but a multitude of different diseases best defined by the specific tumor genetic changes that are driving tumor growth and that can serve as targets for therapy. Although much of the focus to date has been on adenocarcinoma and never-to-light smokers, targets in squamous histology and smokers will need to be developed to make a broader impact on NSCLC. The current state of knowledge of the genomic alterations in squamous cell lung cancer lags behind what is known in adenocarcinoma, but as more attention is focused on this topic, we expect that new targeted therapies for this population will be developed at a rapid pace. There are already several potential targetable mutations that are being actively pursued in clinical trials, and the upcoming Cancer Genome Atlas analysis in squamous cell should also provide a wealth of new information. The Lung Cancer Mutation Consortium, a collaboration of clinical genotyping efforts in lung adenocarcinomas across a host of different academic institutions in the United States, provides a model for genotyping and genotype-driven clinical trial development. Similar focus in squamous cell lung cancer would be invaluable in spurring on the development of targeted therapies in this area.

Acknowledgments

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FIGURE 1.
Interrelationship of signaling pathways involved in genomic alterations seen in squamous cell lung cancer.
# TABLE 1

**Key Candidate Genes in Squamous Cell Lung Cancer**

<table>
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<th>Gene</th>
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<th>Mutation</th>
<th>Level of Evidence</th>
<th>Presence in Other Cancers</th>
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<td>MDM2</td>
<td>10%</td>
<td>5%</td>
<td>1,2</td>
<td>Sarcoma, esophageal, brain, breast, H&amp;N, testicular</td>
<td>84,85</td>
</tr>
<tr>
<td>PDGFRA</td>
<td>8–10%</td>
<td>3–7%</td>
<td>1,2</td>
<td>GBM</td>
<td>76</td>
</tr>
<tr>
<td>MET</td>
<td>6%</td>
<td>&lt;5%</td>
<td>1,2,3</td>
<td>Gastric, esophageal</td>
<td>74</td>
</tr>
<tr>
<td>P53</td>
<td>65%</td>
<td>40%</td>
<td>1,2</td>
<td>Most commonly mutated gene across all cancers</td>
<td>78–80</td>
</tr>
<tr>
<td>NRF2</td>
<td>10–15%</td>
<td>1–2%</td>
<td>1,2</td>
<td>H&amp;N, esophageal, squamous cell of skin</td>
<td>114,115</td>
</tr>
<tr>
<td>PTEN</td>
<td>10.2%</td>
<td>1.7%</td>
<td>1,2</td>
<td>GBM, prostate, breast, gastric, melanoma, endometrial, bladder</td>
<td>111–113</td>
</tr>
<tr>
<td>EPHA2</td>
<td>7%</td>
<td>0</td>
<td>1,2</td>
<td>Breast, prostate, ovary, esophagus, pancreas</td>
<td>69</td>
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<tr>
<td>LKB1</td>
<td>5%</td>
<td>13%</td>
<td>1,2</td>
<td>Cervical</td>
<td>108,109</td>
</tr>
<tr>
<td>AKT</td>
<td>5%</td>
<td>0</td>
<td>1,2</td>
<td>Breast, CRC, ovarian</td>
<td>86,87</td>
</tr>
<tr>
<td>EGFR vIII</td>
<td>5%</td>
<td>0</td>
<td>1,2</td>
<td>GBM</td>
<td>94–96</td>
</tr>
<tr>
<td>DDR2</td>
<td>3–4%</td>
<td>n.r.</td>
<td>1,2</td>
<td>GBM, prostate, breast, gastric, melanoma, thyroid, bladder</td>
<td>111,112</td>
</tr>
<tr>
<td>PTEN loss</td>
<td>IHC expression 70–75%</td>
<td>hypermethylation 30–35%</td>
<td>LOH 18–22% not significantly different by histology</td>
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</table>

**Level of evidence:**

Genomic change reported in primary tumors and cell lines: 1

Functional studies performed showing proliferative effects: 2

Clinical trials demonstrating ability to target genetic alteration and inhibit tumor growth in patients: 3

n.r., not reported; CRC, colorectal cancer; GBM, glioblastoma; HCC, hepatocellular carcinoma; H&N, head and neck cancer.
## TABLE 2

Selected Drugs Targeting Potential Oncogenic Drivers

<table>
<thead>
<tr>
<th>TARGET</th>
<th>Drug</th>
<th>Company</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3K</td>
<td>GDC0032</td>
<td>Genentech</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>ZSTK474</td>
<td>Zenyaku Kogyo</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>GDC0941</td>
<td>Genentech</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>BYL719</td>
<td>Novartis</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>AMG319</td>
<td>Amgen</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>INK1117</td>
<td>Intellikine</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>BKM120</td>
<td>Novartis</td>
<td>I, II</td>
</tr>
<tr>
<td></td>
<td>CAL101</td>
<td>Gilead</td>
<td>I,II</td>
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<tr>
<td></td>
<td>PX-866</td>
<td>Oncothyreon</td>
<td>I,II</td>
</tr>
<tr>
<td></td>
<td>Xi147 SAR245408</td>
<td>Sanofi-Aventis</td>
<td>I, II</td>
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<td>PI3K/MTOR</td>
<td>GSK2126458</td>
<td>GSK</td>
<td>I</td>
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<td></td>
<td>PF-04691502</td>
<td>Pfizer</td>
<td>I</td>
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<tr>
<td></td>
<td>DS-7423</td>
<td>Daichii-Sanyko</td>
<td>I</td>
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<tr>
<td></td>
<td>GDC0980</td>
<td>Genentech</td>
<td>I</td>
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<td>Bay806946</td>
<td>Bayer</td>
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<td>PKI587</td>
<td>Pfizer</td>
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<td>SF1126</td>
<td>Semafore</td>
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<td>BEZ235</td>
<td>Novartis</td>
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<td>FGFR</td>
<td>E-3810</td>
<td>EOS</td>
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<td></td>
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<td>AZ</td>
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<td>Brivanib</td>
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<td>Dasatinib</td>
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<td>INC280</td>
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<td>EMD 1214063</td>
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<td>AXL1717</td>
<td>Axelar AB</td>
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<td>Figutumumab</td>
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</table>

Abstracted from clinicaltrials.gov.

"α" Alpha selective.