Genomics of Squamous Cell Lung Cancer

MELISSA ROONEY,^{a,b} SIDDHARTHA DEVARAKONDA,^c RAMASWAMY GOVINDAN^{a,b}

^aDivision of Oncology, Department of Medicine and ^bAlvin J. Siteman Cancer Center, Washington University School of Medicine, St. Louis, Missouri, USA; ^cDepartment of Internal Medicine, St. Luke's Hospital, Chesterfield, Missouri, USA

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Learning Objectives

Describe important molecular aberrations associated with squamous cell carcinoma.

Identify molecular aberrations that may have therapeutic implications.

ABSTRACT

Approximately 30% of patients with non-small cell lung cancer have the squamous cell carcinoma (SQCC) histological subtype. Although targeted therapies have improved outcomes in patients with adenocarcinoma, no agents are currently approved specifically for use in SQCC. The Cancer Genome Atlas (TCGA) recently published the results of comprehensive genomic analyses of tumor samples from 178 patients with

SQCC of the lung. In this review, we briefly discuss key molecular aberrations reported by TCGA and other investigators and their potential therapeutic implications. Carefully designed preclinical and clinical studies based on these large-scale genomic analyses are critical to improve the outcomes of patients with SQCC of lung in the near future. *The Oncologist* 2013;18:707–716

Implications for Practice: Research conducted over the past few decades has provided crucial insights into the molecular pathogenesis of squamous cell carcinoma (SQCC) of the lung. The ongoing efforts by The Cancer Genome Atlas (TCGA) to comprehensively characterize the genomic landscape of SQCC of the lung has provided new impetus to develop innovative therapeutic approaches.

INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality in the United States and worldwide [1, 2]. It is estimated that lung cancer will account for more than 25% of cancer-related deaths in the United States in the year 2013 [3]. Non-small cell lung cancer (NSCLC) comprises approximately 85% of all lung cancer diagnoses [4, 5]. Squamous cell carcinoma (SQCC) represents approximately 20%–30% of NSCLC cases [6]. Histologically, SQCC is characterized by the presence of keratinization in the form of squamous pearls and intercellular bridges. Similar to cervical cancer, it is thought that SQCCs of the lung develop through a process of dysplasia, progressing into invasive cancers over several years [7]. The World Health Organization currently recognizes four variants of SQCC based on the histological appearance (papillary, clear cell, small cell, and basaloid), although the clinical significance of this classification remains uncertain [6].

The prognosis of patients with metastatic SQCC of the lung continues to be dismal. It is clear that cytotoxic chemotherapy regimens produce only limited benefit in this setting. Novel approaches are urgently needed to significantly improve the outcomes. The massive effort by The Cancer Genome Atlas (TCGA) to understand the genomic landscape of malignant tumors, including SQCC of the lung, has provided insight into several crucial signaling pathways that contribute to the

pathogenesis of cancer. Sixty-four percent of the samples analyzed by TCGA were considered to harbor potentially targetable genetic alterations (Fig. 1) [8]. A number of preclinical and clinical studies are required to translate these exciting findings to improve the overall survival of patients with SQCC of the lung. We review here the findings from the TCGA and other groups that provide rationale for developing innovative clinical trials.

MOLECULAR ALTERATIONS IN SQUAMOUS CELL LUNG CANCER

Chromosomal Level Alterations

Allelic losses that involve loci containing tumor suppressor genes or gains involving selective chromosomal regions that include oncogenes predispose cells to malignant transformation [9]. Haploinsufficiency or partial inactivation of tumor suppressor genes also contributes to tumorigenesis [9, 10]. The most frequently reported sites of allelic loss in SQCC of the lung involve chromosomes 3, 5, 9, 13, and 17 [11–13]. Many of these regions carry certain known tumor suppressor genes, such as *TP53* (17p), *RB1* (13q), and *APC* (5q). In the context of SQCC, amplification of 3q and allelic losses on chromosomal regions 3p and 9p are of particular interest.

Correspondence: Ramaswamy Govindan, M.D., Division of Medical Oncology, Washington University School of Medicine, 660 S. Euclid, Box 8056, St. Louis, Missouri 63021, USA. Telephone: 314-362-5737; Fax: 314-362-4232; E-Mail: rgovinda@dom.wustl.edu Received February 27, 2013; accepted for publication April 24, 2013; first published online in *The Oncologist Express* on May 31, 2013. ©AlphaMed Press 1083-7159/2013/\$20.00/0 <http://dx.doi.org/10.1634/theoncologist.2013-0063>

Figure 1. Druggable genetic alterations in squamous cell carcinoma: The Cancer Genome Atlas data. Reprinted from [8] with permission. Abbreviation: MA F impact: Functional impact of mutation determined by Mutation Assessor score.

Figure 2. Schematic representation of possible genetic alterations that correlate with SQCC progression [6, 13, 16, 139]. Adapted from Wistuba et al. and Drilon et al. Abbreviation: LOH: loss of heterozygosity.

Chromosomal Region 3q

Evidence from previous studies, including the recently published TCGA study, strongly suggests a role for selective amplification of 3q in the pathogenesis of SQCC (Fig. 2) [8]. Genomic gain at this region has been considered to be among the most prevalent and significant molecular aberrations in SQCC [14]. Gains in this region were observed in as many as 86% of SQCC samples (19 out of 22) but only 21% of adenocarcinoma samples (3 out of 14) in a study by Kang et al. [15]. In a longitudinal bronchoscopic surveillance study, patients with premalignant bronchial dysplastic lesions were serially biopsied. None of the patients with low-grade lesions demonstrated copy number changes in the 3q region, whereas all patients with high-grade lesions demonstrated 3q amplification [16]. The majority of patients with high-grade lesions and 3q amplification developed invasive cancer in this study. Genes of in-

terest in the 3q region include *SOX2*, *TP63*, *PIK3CA*, and *EPHB3* [15, 17].

Chromosomal Region 3p

Allelic losses on the 3p region have been frequently reported in lung cancer [18, 19]. Tumor suppressor genes such as *RASSF1A*, *FUS1*, *VHL*, and *FHIT* have been mapped to this region. The extent of allelic losses at this region has been observed to be greater in tumors with a squamous histology compared with adenocarcinoma, as well as in early stages of disease development [12, 19]. Wistuba et al. analyzed the 3p region for loss of heterozygosity with a panel of 28 markers; they reported allelic losses in 78% of preneoplastic (54 samples) and 96% of lung cancer samples (in a total of 97 samples with 2 SQCC cell lines and 23 SQCC samples) [12]. In another study that assessed 3p-specific epigenetic alterations, SQCC

Comprehensive evaluation of somatic copy number alterations (SCNAs) of SQCC samples using SNP arrays revealed an average of 323 SCNAs per sample. Of these alterations, selective amplification of 50 genes was considered significant.

samples were more frequently found to be hypermethylated at this region compared with adenocarcinomas [20].

Chromosome Region 9p

Deletions involving chromosomal loci 9p21.3, 9p24.1, and 9p13.1 in SQCC were reported by TCGA [8]. These loci harbor tumor suppressor genes such as *CDKN2A* and *PTPRD* [21]. Loss of heterozygosity at the locus of *CDKN2A* has been observed early in the development of lung cancer (Fig. 2) [11]. This gene is known to code for two critical cell cycle regulating proteins by alternate splicing: p16INK4a and p14ARF [22, 23]. Evidence that supports the role of *CDKN2A* in the early pathogenesis of SQCC originates from studies that have described epigenetic inactivation of p16INK4a in SQCC, especially in smokers (current or former) [24, 25].

Belinsky et al. also reported the frequency of p16INK4a promoter hypermethylation to correlate with histological progression of SQCC (17% in basal cell hyperplasia, 24% in squamous metaplasia, and 50% in carcinoma in situ) [24]. Inactivation of *CDKN2A* by methylation, inactivating mutations, exon 1 β skipping, or homozygous deletions was observed in 72% of the 178 SQCC samples analyzed by TCGA [8]. *PTPRD* is a protein tyrosine phosphatase that plays a crucial role in STAT3 signaling and is considered to be a frequent target of inactivation in several cancers, including glioblastomas, SQCC of the head and neck and lung cancer [26].

Somatic Copy Number Alterations

Comprehensive evaluation of somatic copy number alterations (SCNAs) of SQCC samples using SNP arrays revealed an average of 323 SCNAs per sample [8]. Of these alterations, selective amplification of 50 genes was considered significant. Some of the genes affected by such SCNAs and their presumed role in oncogenesis are briefly reviewed here.

SOX2

The sex determining region Y (SRY)-box 2 gene (*SOX2*) lies on 3q and is believed to be a key oncogene in this region [16]. *SOX2* participates in lung organogenesis. Mice that overexpress *SOX2* demonstrate abnormal branching of the lung [27]. Overexpression of *SOX2* drives cells to prematurely commit to either neuroendocrine (cGRP positive) or prebasal (delta-Np63 positive) lineages and promotes coexpression of squamous markers such as p63 and keratin 6, suggesting a role for *SOX2* in squamous differentiation [28]. These observations support a role for *SOX2* as a “lineage survival oncogene” in SQCC, in which lineage-specific survival and differentiation programs are exploited by the cancer cell [29, 30]. Evidence from cell line studies indicates that *SOX2* overexpression drives anchorage independent growth and invasion in cancer cells, whereas knockdown or underexpression inhibits cell growth and metastatic potential [29, 31].

SOX2 also facilitates reprogramming of differentiated cells into pluripotent cells [33, 34]. Overexpression of *SOX2* has been proposed to maintain some of the characteristics of cancer cells [28, 34]. Nearly 20% of lung SQCC samples have been found to have *SOX2* amplification [29, 32]. Squamous differentiation pathway was altered in 44% and *SOX2* was activated in 21% of the samples analyzed by TCGA [8].

FOXP1

FOXP1 plays an important role in lung and esophageal development [35]. FOX family of proteins are highly conserved with well-defined roles in cancer [36]. The *FOXP1* gene is located in 3p, a region commonly deleted in SQCC of the lung [12, 37]. Both tumor promoting and suppressor roles have been attributed to *FOXP1*. Overexpression of *FOXP1* in NSCLC has been associated with better survival in patients with lung cancer [38]. Messenger RNA (mRNA) levels of *FOXP1* were reported to be lower in SQCC than adenocarcinoma samples at a significant level in a study conducted by Dmitriev et al. [20]. Being a lineage-associated gene and a direct target of *SOX2* repression, it is possible that downregulation of *FOXP1* plays an important role in dedifferentiation [28]. Inactivation of *FOXP1* was reported in 4% of the samples analyzed by TCGA [8].

PDGFRA

In a study carried out by Ramos et al., amplifications of the chromosomal region 4q12 were observed in 8.7% (5 of 57) of lung SQCC samples [39]. This region harbors the genes *PDGFRA* and *KIT*. *PDGFRA* and *KIT* are frequently mutated in gastrointestinal stromal tumors (GISTs) and activating mutations in *KIT* are considered targetable by small molecule inhibitors such as imatinib [40]. Knockdown of *PDGFRA* expression by small hairpin RNA resulted in decreased cell survival and growth in a NSCLC cell line (NCI-H1703) overexpressing *PDGFRA*, when compared to a control cell line (HCC15) [39]. Amplification of *PDGFRA* was observed in 4% of the cases by TCGA [8, 41]. *PDGFRA* is a target of several multitargeted tyrosine kinase inhibitors, including sunitinib, pazopanib, cediranib, and nintedanib, which are currently in clinical development for NSCLC.

Sunitinib, a multitargeted tyrosine kinase inhibitor, is approved by the U.S. Food and Drug Administration for the treatment of imatinib-resistant GISTs, pancreatic neuroendocrine tumors, and advanced renal cell carcinoma; currently, it is being investigated in NSCLC. Two phase II trials investigating the use of second-line sunitinib in advanced NSCLC have shown modest benefit in NSCLC. Socinski et al. studied sunitinib 50 mg daily for 4 weeks, followed by 2 weeks off, in patients with advanced NSCLC who had progressed on prior platinum therapy; the authors reported an overall response rate (ORR) of 11.1% (95% confidence interval [CI]: 4.6%–21.6%), a median progression-free survival (PFS) time of 12 weeks (95% CI: 10–16.1 weeks), and a median overall survival (OS) time of 23.4 weeks (95% CI: 17.0 to 28.3 weeks) [42].

Novello et al. investigated continuous daily sunitinib (37.5 mg daily) in patients who had progressed on previous platinum therapy and found an ORR of 2.1% (95% CI: 0.1%–11.3%), a median PFS of 11.9 weeks (95% CI: 8.6–14.1 weeks), and median OS of 37.1 weeks (95% CI: 31.1–69.7 weeks) [43]. Sunitinib maintenance therapy is currently being investigated

in a phase III trial conducted by the ALLIANCE cooperative group (CALGB 30607).

Pazopanib, a multitargeted tyrosine kinase inhibitor, is currently being studied in phase II/III second-line studies for advanced NSCLC as monotherapy (EORTC 0809, NCT01049776) and in first-line combination therapy in patients ineligible for platinum doublet therapy (NCT01179269). In a preoperative study, pazopanib produced notable reduction in tumor volume in 30 out of 35 patients [44].

A phase II/III randomized double-blinded study was performed using carboplatin and paclitaxel in combination with cediranib (30 mg) or placebo in advanced NSCLC in the first-line setting. The phase II analysis revealed a higher response rate for cediranib versus placebo (38 vs. 16%; $p < .001$) and hazard ratio (HR) for PFS of 0.77 (99% CI: 0.56–1.08) [45]. However, patients in the cediranib arm had more reported toxicities, including hypertension, hypothyroidism, and gastrointestinal toxicity.

Nintedanib (BIBF 1120) is a tyrosine kinase inhibitor with activity against vascular endothelial growth factor receptors (VEGFRs), platelet-derived growth factor receptor (PDGFRs), and fibroblast growth factor receptors (FGFRs). Okamoto et al. performed a phase I study of advanced solid tumors and established a maximum tolerated dose of 200 mg b.i.d. and observed stable disease in 76.2% of patients enrolled [46]. Nintedanib was found to be tolerated well when administered in combination with pemetrexed in a phase I study [47]. One complete response was noted and 50% of patients enrolled achieved stable disease as best overall response.

In a phase II clinical trial, two doses of single-agent nintedanib (150 mg b.i.d. versus 250 mg b.i.d.) were compared in patients with advanced NSCLC who had progressed on platinum-based chemotherapy [48]. Median PFS was 6.9 weeks and median OS time was 21.9 weeks. Outcomes were similar in both arms. Patients with an Eastern Cooperative Oncology Group performance status of 0 or 1 had an improved median PFS of 11.6 weeks and median OS of 37.7 weeks. Results from randomized trials with this agent are expected soon. Although these agents have modest activity in metastatic NSCLC, whether they will have activity in a defined subgroup of patients with activation of *PDGFR* or other related genes remains to be tested.

NFE2L2, KEAP1, and CUL3

NFE2L2 encodes the NRF2 transcription factor (nuclear factor erythroid-related factor 2). NRF2 is upregulated during times of stress and facilitates production of antioxidants, drug efflux pumps, and enzymes that mediate xenobiotic detoxification [49, 50]. Overexpression of NRF2 thus enables cancer cells to survive hostile environments and evade therapy [51, 52].

KEAP1 (Kelch-like ECH associated protein 1) negatively regulates the activity of NRF2 by facilitating its degradation [53]. Singh et al. reported on functionally important mutations in the Kelch and intervening regions of the *KEAP1* protein as well as frequent biallelic inactivation of *KEAP1* in the context of NSCLC [53].

CUL3 (Cullin 3), a member of the E3 ligase complex, is also responsible for mediating the degradation of NRF2 [54]. De-

creased activity of KEAP1 or CUL3 can result in intracellular accumulation of NRF2 [8]. Overall, alterations in the NRF2-mediated oxidative stress pathway were present in 34% of SQCC samples analyzed by TCGA. Solis et al. studied the immunohistochemical expression of NRF2 and KEAP1 in 304 NSCLC samples and correlated increased expression of NRF2 in NSCLC ($p = .0139$, HR: 1.75) and decreased expression of KEAP1 in SQCC ($p = .0181$, HR: 2.09) with worse overall survival [55].

EPHB3

The Eph-ephrin signaling system constitutes membrane-bound tyrosine kinase receptors and ligands that are capable of participating in reverse signaling [56]. Eph-ephrin signaling system mediates several key functions, such as guiding axonal growth and angiogenesis during embryogenesis. Both tumor suppressor and promoting roles have been attributed to these genes and their roles in lung and other cancers has been reviewed elaborately [56–58].

Among several members of the Eph-ephrin family, *EPHB3*, *EPHA2*, and *EPHA3* are frequently altered in lung cancer [8, 57, 59–64]. Overexpression of *EPHB3* has been reported in NSCLC and is associated with the promotion of a metastatic phenotype in these tumors. The ability of *EPHB3* to drive metastasis is believed to be independent of its kinase activity [64, 65]. *EPHB3* was amplified in 37% of SQCC samples evaluated by the TCGA [8, 41]. Stahl et al. reported a possible role for *EPHB3* expression in mediating resistance to radiotherapy [66]. *EPHB3* inhibition by siRNA also was demonstrated to increase radiation sensitivity of NSCLC cells in vitro. Given that the Eph-ephrin family of molecules is overexpressed across a variety of cancers, several molecules that target this signaling system are currently being investigated [56–58]. Kinase inhibitors capable of interfering with the tumor promoting effects of Eph-ephrin signaling, peptides and antibodies capable of inhibiting Eph-ephrin interactions, and vaccines and immunotherapies capable of facilitating tumor lysis or rejection are some of the strategies that have been described to date [67–72]. Data on the safety and efficacy of these agents in lung cancer is very limited. Agents such as XL647 and dasatinib, which are multi-kinase inhibitors capable of targeting Eph receptors as well, have not yet shown promising results in NSCLC [68, 73]. A better understanding of Eph/ephrin kinase signaling within the cancer cell and tumor microenvironment is necessary to develop effective targeted therapies.

BCL2L1

BCL2L1 is a pro-survival, BH3 domain-containing protein that plays a crucial role in inhibiting cellular apoptosis [74, 75]. Amplification of *BCL2L1* was present in a small number (about 5%) of the SQCC samples studied by TCGA [8].

Mutations

SQCC of the lung is known to carry a very high number of protein-altering mutations [76]. A total of 48,690 nonsilent mutations were reported in 18,863 genes by TCGA in 178 SQCC tumor samples [8]. This accounted for an average of 228 nonsilent mutations per sample and a mutation rate of 8.1/Mb (mega base) of DNA. Of these, 10 genes (*TP53*, *CDKN2A*, *PTEN*, *PIK3CA*, *KEAP1*, *MLL2*, *HLA-A*, *NFE2L2*, *NOTCH1*, and *RB1*) were considered to be significantly mutated. In another study

that sequenced selected oncogenes in NSCLC, mutations in *DDR2* and *FGFR2* were reported to be restricted to smokers with SQCC [77]. In the same study, *EGFR*, *MET*, and *PIK3CA* mutations were more prevalent in nonsmokers with SQCC compared with smokers [77]. Some of these frequently mutated genes and their role in the pathogenesis of SQCC will be briefly reviewed here.

TP53, MDM2, and RB1

TP53, a tumor suppressor gene located on chromosome 17, is perhaps the most studied gene in cancer. The p53 protein is instrumental in regulating the cell cycle and apoptosis. Loss of p53 expression has been described in almost all cancer types and the TCGA reported a mutation rate of approximately 90% in SQCC [8]. MDM2 is a protein that negatively regulates p53 by blocking its N-terminal activating domain [78]. Overexpression of MDM2 has been described in multiple tumors, including small cell lung cancer, and this gene is also considered to be potentially targetable [79]. *RB1*, a cell cycle-regulating gene like *TP53*, has been implicated in the development of multiple cancers; inactivation has been reported in 7% of SQCC samples analyzed by TCGA.

TP63

TP63 encodes two isoforms by differential splicing that take part in contrasting cell processes [17, 80]. The TA-p63 isoform largely functions as a tumor suppressor, whereas the deltaN-p63 (also called p40) isoform is pro-oncogenic. The p40 is a truncated isoform of p63 and facilitates dominant-negative inactivation of tumor suppressor proteins p53 and TA-p63. Pluripotent basal cells in the epidermis typically demonstrate intense staining for this isoform [81]. Because of its ability to inhibit p53, it is believed that p40 aids in the maintenance of pluripotency and a constant stem cell population in basal progenitor cells [82]. The TCGA project identified p40 as the dominant isoform expressed (89%) in SQCC compared to TA-p63.

PIK3CA

PIK3CA is one of the most frequently altered genes in cancer [75, 83, 84]. Activation of the PI3K kinase signaling system results in AKT activation and enables cancer cells to acquire multiple “hallmark” characteristics [85]. In cell line studies with immortalized airway cells, overexpression of wild-type *PIK3CA* was shown to promote anchorage independent growth and migration [86]. Missense mutations of the *PIK3CA* gene, particularly at positions 545 and 1047 (involving the helicase and kinase domains), demonstrated a similar behavior in these cells. Evidence from studies in mice also suggests a crucial metastasis-enabling role for *PIK3CA* [87]. *PIK3CA* amplification is more frequently associated with patients with squamous histology, men, and smokers [88, 89].

PIK3CA was one of the significantly mutated genes in the TCGA SQCC project [8]. Interestingly, 12 of the 28 SQCC samples with *PIK3CA* mutations in this study carried mutations at positions 545 and 1047. Further evidence to support the role of *PIK3CA* in SQCC also comes from studies examining *PTEN*—a gene that regulates the *PIK3CA* signaling cascade and is significantly altered in these tumors [8].

PTEN

The *PTEN* gene is a tumor suppressor that is frequently altered in cancer [75]. *PTEN* catalyzes the dephosphorylation and inactivation of intracellular signaling molecule phosphatidylinositol-3,4,5-trisphosphate (*PIP3*), and inactivation of *PTEN* results in the increase in AKT signaling [85]. *PTEN* and *TP53* are often co-repressed in NSCLC [90]. Inactivation of *PTEN* is common in SQCC and occurs early in the disease [91]. TCGA reported *PTEN* alterations in 15% of the samples and these alterations were more frequently associated with the classical and primitive subtypes (based on expression profiling) [8].

Loss of *PTEN* can disrupt negative feedback on cell proliferation and facilitate metastasis by affecting FAK/CDC42RhoGTPase-based pathways, which are capable of driving cell proliferation, adhesion, and migration [92, 93]. Trials evaluating agents that inhibit *PIK3CA* and AKT are at various stages of clinical development. A phase II study of the PI3K inhibitor BKM 120 in pretreated metastatic NSCLC displaying PI3K pathway is currently underway (NCT01297491). A phase I/II study of BKM 120 in combination with every-3-week carboplatin and paclitaxel is also under investigation for the treatment of metastatic SQCC (NCT01820325).

EGFR

EGFR mutations were more frequent in SQCC tumor samples from nonsmokers compared with those from smokers (8% vs. 2.1%) [77]. Unlike adenocarcinoma, exon 19 deletions and exon

Although these studies report an association between *EGFR* mutation status and better outcomes with *EGFR* TKIs, outcomes in *EGFR* TKI-treated SQCC are usually inferior in comparison to adenocarcinomas.

21 mutations are uncommon in SQCC of the lung [94]. Only a limited number of studies have examined the frequency of *EGFR* mutations in SQCC of the lung. *EGFR* mutations involving exon 19 or 21 were present in 15%–46% of lung SQCC samples examined in two small studies from Asia [95, 96]. While no exon 19 deletion or L858R mutations were observed in TCGA study, two samples were found to have *EGFR* mutations (L861Q) known to confer sensitivity to gefitinib or erlotinib [6].

Although these studies report an association between *EGFR* mutation status and better outcomes with *EGFR* TKIs, outcomes in *EGFR* TKI-treated SQCC are usually inferior in comparison to adenocarcinomas [95, 97]. A statistically non-significant correlation between increased survival and combination therapy with cetuximab and chemotherapy was observed in 377 patients with advanced SQCC in the FLEX (First-Line ErbituX in lung cancer) trial (HR for death: 0.8, 95% CI: 0.64–1.00) [98]. However, increased *EGFR* expression levels were considered to correlate with better outcomes in the FLEX trial [99]. A similar association between increased *EGFR* gene copy number and improved outcomes with *EGFR*-targeted therapies has also been reported in other studies [100]. Although *EGFR* amplifications were reported in 7% of the samples analyzed by TCGA, the extent to which these patients will respond to existing *EGFR*-targeted therapies is uncertain at this time [8, 99, 100].

The *EGFR* vIII mutation, commonly associated with gliomas, has also been reported in about 5% of the SQCC samples analyzed by Ji et al. [101, 102]. This variant is characterized by the deletion of exons 2–7 that prevents ligand binding but confers constitutively active kinase activity to the receptor [103]. Decreased response to therapy with cisplatin and cetuximab was reported in head and neck SQCCs (HNSCCs) harboring this variant [104]. Evidence from a cell line study also suggests that *EGFR* vIII harboring tumors are relatively resistant (>40 fold more resistant compared with L858R variant) to gefitinib and erlotinib [102]. Although strategies capable of targeting this variant have been described in the literature, none are currently available for clinical use [105]. Trials investigating several existing and next-generation agents capable of inhibiting *EGFR* signaling are currently underway (Table 1).

DDR2

DDR2 (discoidin domain containing receptor 2), a tyrosine kinase receptor that binds fibrillar collagen, was recently reported as a potentially targetable mutation in SQCC of the lung, with an estimated frequency close to 4% [106]. Pathways downstream of *DDR2* regulate cell differentiation, proliferation, and migration. Hammerman et al. tested several multikinase inhibitors and found that the use of dasatinib facilitated cell death in *DDR2*-mutated SQCC cell lines [106]. A phase II study to evaluate the efficacy of dasatinib in advanced SQCC of the lung (NCT01491633) was recently closed because of toxicity issues. Novel *DDR2* inhibitors with better safety profiles are urgently required.

FGFR1

FGFR1 is a tyrosine receptor kinase that specifically binds ligands of the fibroblast growth factor family and is responsible for inducing mitosis, cell differentiation, and angiogenesis [107]. Preclinical studies have demonstrated *FGFR1* amplification predominantly involving SQCC of the lung in current smokers [108]. Overexpression of *FGFR1* has been reported in 9.7%–21% of SQCC samples [108, 109]. Activation of *FGFR1* was present in 7% of the samples analyzed by TCGA [8]. Weiss et al. described the use of a small molecule PD173074 that nonspecifically blocks the FGF family of receptors. In their study, PD173074 was able to inhibit tumor growth and promote apoptosis in cells overexpressing *FGFR1*. These findings suggest that the blockade of *FGFR1* may be a promising target in treating SQCC. Multiple *FGFR* inhibitors are in early clinical development [107]. BGJ398 and AZD4546 are pan-*FGFR* kinase inhibitors currently being studied in the phase I setting. Multikinase inhibitors targeting *FGFR* in combination with *VEGFR* and *PDGFR* are also in early phases of clinical testing, including E-3810, a dual *VEGFR*-*FGFR* tyrosine kinase inhibitor [110].

ERBB2

ERBB2 is a proto-oncogene located on the long arm of chromosome 17 (17q12) and encodes HER2/Neu, a receptor tyrosine kinase of the epidermal growth factor receptor family. Overexpression of *HER2* contributes to the pathogenesis of many cancers including breast, ovarian, gastric, and salivary gland cancers [111–114]. Stephens et al. reported mutations in *ERBB2* kinase domain in 4% of the tumor samples from pa-

tients with lung cancer [115]. All patients with *ERBB2* mutations had adenocarcinoma histology. Evidence suggests a role for *ERBB2* signaling in mediating resistance of lung cancers to *EGFR*-targeting therapies [116]. Given that mutation and amplification of *ERBB2* in SQCC were reported in 3% and 2% of the samples analyzed by TCGA, respectively, therapy targeting *ERBB2* could potentially benefit a subset of patients with SQCC [8, 41].

NF1

The neurofibromin 1 (*NF1*) gene is located on the chromosomal region 17q. This gene plays an important role in the development of epidermal cells [117]. It is often expressed in basal keratinocytes during the fetal period. *NF1* plays the role of a tumor suppressor protein by inhibiting RAS. Mutations in *NF1* have been reported in both NSCLC and small cell lung cancer [61, 118, 119]. It is possible that *NF1* plays an important role in the pathogenesis of SQCC given that it is required for the normal development of epidermal cells. *NF1* was inactivated in 11% of the samples examined by TCGA [8]. More studies are definitely required to establish the role of *NF1* in SQCC.

NOTCH1

NOTCH signaling pathways are important for embryogenesis and controlling cell differentiation. Mutations in the NOTCH signaling pathway have been described in the development of many cancers [120]. Activating mutations in the *NOTCH1* gene have been implicated in more than 50% of all cases of T-cell acute lymphoblastic leukemia [121]. Inhibition of NOTCH signaling has antiproliferative effects in mouse models [122]. Loss of function in the NOTCH signaling pathway secondary to truncating mutations of *NOTCH1* have been described in HNSCC [123]. TCGA investigators reported *NOTCH1* mutations in 8% of SQCCs of the lung [8]. Of these mutations, 8 of 17 were found to be truncating mutations similar to those described in SQCC of the head and neck.

HLA-A

Human leukocyte antigen A (*HLA-A*) is encoded by the *HLA-A* locus on chromosome 6p. *HLA-A* is part of the major histocompatibility complex class I, which functions as part of the immune system to present foreign polypeptides to the immune system and specifically target cells for destruction by activation of T lymphocytes [124]. Loss of function mutations within the *HLA-A* gene locus in SQCC, primarily through nonsense or splicing mutations, were reported for the first time by TCGA [8]. These mutations in the *HLA-A* gene suggest a possible mechanism by which tumor cells can successfully evade immune destruction and present a potential role for immunotherapy.

MLL2

Mixed lineage leukemia 2 (*MLL2*) is a member of the trithorax family of human genes, encoding a histone-lysine-N-methyltransferase on chromosome 12q13.12 [125]. *MLL2* is involved in epigenetic programming and embryonic development and is overexpressed in several malignancies such as medulloblastoma, breast cancer, colon cancer, and non-Hodgkin's lymphoma [126–128]. *MLL2* was reported as significantly mutated in SQCC of the lung with 7 silent mutations, 40 nonsilent mutations, and 18 frame shift and

Table 1. Some of the actively recruiting clinical trials testing targeted agents in squamous cell carcinoma of the lung

Target	Drug	Clinicaltrials.gov numbers
<i>EGFR</i>	Erlotinib, panitumumab, gefitinib, afatinib, cetuximab, RO5083945	NCT00979212, NCT00661193, NCT01294306, NCT01059188, NCT00966472, NCT01013831, NCT00835471, NCT010204697, NCT01702714, NCT01485809, NCT01523587, NCT00946712
Angiogenesis	Bevacizumab, ramucirumab, NGR-hTNF, nintedanib	NCT00946712, NCT01160744, NCT00994097, NCT01346540
<i>AKT</i>	MK2206	NCT01294306
<i>PI3K</i>	PX866, BKM120	NCT01204099, NCT01297491
<i>ERBB</i>	Dacomitinib (pan-ErbB), M121 (ErbB3)	NCT01441128, NCT01451632
Other	Dasatinib (DDR2), crizotinib (EML4-ALK)	NCT01491633, NCT01514864, NCT01441128

Data is from the ClinicalTrials.gov database, accessed November 5, 2012.

nonsense mutations by TCGA [8]. Several emerging therapeutic strategies targeting epigenetic pathways are currently being investigated [129, 130].

mRNA EXPRESSION PROFILING

Using expression arrays, Wilkerson et al. identified four mRNA expression subtypes of SQCC of the lung: classical, primitive, basal, and secretory [131]. These findings were replicated in the SQCC TCGA study [8]. The classical subtype was the most prevalent (36% of TCGA samples) and was predominantly encountered in men, smokers, and tumors overexpressing genes responsible for xenobiotic metabolism and those on 3q including *SOX2*, *TP63*, and *PIK3CA*. The primitive type, accounting for 15% of all TCGA samples, was characterized by rapid cell proliferation, poor differentiation, and worse overall survival. The basal subtype (25%), in contrast to the primitive type, was characterized by a well-differentiated phenotype. Genes altered in basal tumors played an important role in cell adhesion and epidermal development. Lastly, the secretory type (24%) was associated with alteration in genes responsible for mediating immune responses as well as mucin and surfactant production. The clinical implications of these findings need to be explored in larger groups of patients with SQCC of the lung.

STRUCTURAL VARIATIONS

Translocations involving *ALK*, *RET*, and *ROS1* have been reported in NSCLC (mostly adenocarcinoma) and are amenable for therapy with targeted agents such as crizotinib [132–135]. On average, 165 somatic rearrangements per sample were identified in the tumor samples in the SQCC TCGA project [8]. None of these events were recurrent or associated with a fusion protein. Additional work needs to be done to understand the significance of these findings.

EPIGENETIC ALTERATIONS

Epigenetic alteration by differential methylation of genes is common in SQCC [136]. Hypermethylated differentially methylated regions (DMRs) were overrepresented in SQCC samples on genome-wide DNA methylation analysis of NSCLC samples [136]. Of these, 287 DMRs were found to be unique to SQCC, whereas only 26 DMRs were unique for adenocarcinomas. Although there was heterogeneity in the functions of these hypermethylated genes, genes associated with the regulation of transcription, cytoskeletal organization, and cell cycle were overrepresented in these

samples. Promoter hypermethylation was more frequently reported in the classical subtype by TCGA investigators [8, 131]. Hypermethylation of the p16INK4a promoter (especially in smokers) has been discussed previously. Epigenetic changes that bring about the inactivation of *TP53* have also been reported in SQCC [137]. The *HIC1-SIRT1-p53* circular loop deregulation has been described as one way of achieving such an inactivation. Decreased expression of the hypermethylated in cancer (*HIC1*) gene renders it incapable of repressing *SIRT1*, a deacetylase that inactivates *TP53*. Differential methylation patterns of a gene cluster on 9p21 composed of p16INK4a, p15INK4b, and p14ARF promoters between central and peripheral SQCC has also been reported [138]. The significance of these differences is unclear.

SUMMARY

Ongoing efforts by TCGA investigators will map the genomic landscape of 500 tumor samples from patients with SQCC of the lung by the end of this year. These and other large-scale efforts will help us understand the molecular pathogenesis of SQCC of the lung better over the next few years. Carefully designed preclinical and clinical studies to translate these findings in the clinic will very likely improve the outcomes of patients with SQCC of the lung.

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AUTHOR CONTRIBUTIONS

Conception/Design: Ramaswamy Govindan

Collection and/or assembly of data: Melissa Rooney, Siddhartha Devarakonda

Data analysis and interpretation: Melissa Rooney, Siddhartha Devarakonda, Ramaswamy Govindan

Manuscript writing: Melissa Rooney, Siddhartha Devarakonda, Ramaswamy Govindan

Final approval of manuscript: Melissa Rooney, Siddhartha Devarakonda, Ramaswamy Govindan

DISCLOSURES

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