

Comprehensive genomic characterization of squamous cell lung cancers

The Cancer Genome Atlas Research Network*

Lung squamous cell carcinoma is a common type of lung cancer, causing approximately 400,000 deaths per year worldwide. Genomic alterations in squamous cell lung cancers have not been comprehensively characterized, and no molecularly targeted agents have been specifically developed for its treatment. As part of The Cancer Genome Atlas, here we profile 178 lung squamous cell carcinomas to provide a comprehensive landscape of genomic and epigenomic alterations. We show that the tumour type is characterized by complex genomic alterations, with a mean of 360 exonic mutations, 165 genomic rearrangements, and 323 segments of copy number alteration per tumour. We find statistically recurrent mutations in 11 genes, including mutation of *TP53* in nearly all specimens. Previously unreported loss-of-function mutations are seen in the *HLA-A* class I major histocompatibility gene. Significantly altered pathways included *NFE2L2* and *KEAP1* in 34%, squamous differentiation genes in 44%, phosphatidylinositol-3-OH kinase pathway genes in 47%, and *CDKN2A* and *RBI* in 72% of tumours. We identified a potential therapeutic target in most tumours, offering new avenues of investigation for the treatment of squamous cell lung cancers.

Lung cancer is the leading cause of cancer-related mortality worldwide, leading to an estimated 1.4 million deaths in 2010 (ref. 1). The discovery of recurrent mutations in the epidermal growth factor receptor (*EGFR*) kinase, as well as fusions involving anaplastic lymphoma kinase (*ALK*), has led to a marked change in the treatment of patients with lung adenocarcinoma, the most common type of lung cancer^{2–5}. More recent data have suggested that targeting mutations in *BRAF*, *AKT1*, *ERBB2* and *PIK3CA* and fusions that involve *ROS1* and *RET* may also be successful^{6,7}. Unfortunately, activating mutations in *EGFR* and *ALK* fusions are typically not present in the second most common type of lung cancer, lung squamous cell carcinoma (SQCC)⁸, and targeted agents developed for lung adenocarcinoma are largely ineffective against lung SQCC.

Although no comprehensive genomic analysis of lung SQCCs has been reported, single-platform studies have identified regions of somatic copy number alterations in lung SQCCs, including amplification of *SOX2*, *PDGFRA* and *FGFR1* and/or *WHSC1L1* and deletion of *CDKN2A*^{9,10}. DNA sequencing studies of lung SQCCs have reported recurrent mutations in several genes, including *TP53*, *NFE2L2*, *KEAP1*, *BAI3*, *FBXW7*, *GRM8*, *MUC16*, *RUNX1T1*, *STK11* and *ERBB4* (refs 11, 12). *DDR2* mutations and *FGFR1* amplification have been nominated as therapeutic targets^{13–15}.

We have conducted a comprehensive study of lung SQCCs from a large cohort of patients as part of The Cancer Genome Atlas (TCGA) project. The twin aims are to characterize the genomic and epigenomic landscape of lung SQCC and to identify potential opportunities for therapy. We report an integrated analysis based on DNA copy number, somatic exonic mutations, messenger RNA sequencing, mRNA expression and promoter methylation for 178 histopathologically reviewed lung SQCCs, in addition to whole genome sequencing (WGS) of 19 samples and microRNA sequencing of 159 samples (Supplementary Table 1.1). Demographic and clinical data and results of the genomic analyses can be downloaded from the TCGA data portal (https://tcga-data.nci.nih.gov/docs/publications/lusc_2012/).

Samples and clinical data

Tumour samples were obtained from 178 patients with previously untreated stage I–IV lung SQCC. Germline DNA was obtained from

adjacent, histologically normal tissues resected at the time of surgery ($n = 137$) or from peripheral blood ($n = 41$). All patients provided written informed consent to conduct genomic studies in accordance with local Institutional Review Boards. The demographic characteristics are described in Supplementary Table 1.2. The median follow-up for the cohort was 15.8 months, and 60% of patients were alive at the time of the last follow-up (data updated in November 2011). Ninety-six per cent of the patients had a history of tobacco use, similar to previous reports for North American patients with lung SQCC¹⁶. DNA and RNA were extracted from patient specimens and measured by several genomic assays, which included standard quality-control assessments (Supplementary Methods, sections 2–8). A committee of experts in lung cancer pathology performed a further review of all samples to confirm the histological subtype (Supplementary Fig. 1.1 and Supplementary Methods, section 1).

Somatic DNA alterations

The lung SQCCs analysed in this study display a large number and variety of DNA alterations, with a mean of 360 exonic mutations, 323 altered copy number segments and 165 genomic rearrangements per tumour.

Copy number alterations were analysed using several platforms. Analysis of single nucleotide polymorphism (SNP) 6.0 array data across the set of 178 lung SQCCs identified a high rate of copy number alteration (mean of 323 segments) when compared with other TCGA projects (as of 1 February 2012), including ovarian cancer (477 segments)¹⁷, glioblastoma multiforme (282 segments)¹⁸, colorectal carcinoma (213 segments), breast carcinoma (282 segments) and renal cell carcinoma (156 segments) ($P < 1 \times 10^{-15}$ by Fisher's exact test). These segments gave rise to regions of both focal and broad somatic copy number alterations (SCNAs), with a mean of 47 focal and 23 broad events per tumour (broad events defined as $\geq 50\%$ of the length of the chromosome arm). There was strong concordance between the three independent copy number assays for all regions of SCNA (Supplementary Figs 2.1–2.4).

At the level of whole chromosome arm SCNAs, lung SQCCs exhibit many similarities to 205 cases of lung adenocarcinoma analysed by

*Lists of participants and their affiliations appear at the end of the paper.

TCGA (Supplementary Fig. 2.1a). The most notable difference between these cancers is selective amplification of chromosome 3q in lung SQCC, as has been reported^{9,19}. Using the SNP 6.0 array platform and GISTIC 2.0 (refs 20, 21), we identified regions of significant copy number alteration (Supplementary Methods, section 2). There were 50 peaks of significant amplification or deletion ($Q < 0.05$), several of which included SCNAs previously seen in lung SQCCs including *SOX2*, *PDGFRA* and/or *KIT*, *EGFR*, *FGFR1* and/or *WHSC1L1*, *CCND1* and *CDKN2A*^{9,10,19} (Supplementary Fig. 2.1b and Supplementary Data 2.1 and 2.2). Other peaks defined regions of SCNA reported for the first time, including amplifications of chromosomal segments containing *NFE2L2*, *MYC*, *CDK6*, *MDM2*, *BCL2L1* and *EYS* and deletions of *FOXP1*, *PTEN* and *NF1* (Supplementary Fig. 2.1b).

Whole exome sequencing of 178 lung SQCCs and matched germline DNA targeted 193,094 exons from 18,863 genes. The mean sequencing coverage across targeted bases was 121 \times , with 83% of target bases above 30 \times coverage. We identified a total of 48,690 non-silent mutations with a mean of 228 non-silent and 360 total exonic mutations per tumour, corresponding to a mean somatic mutation rate of 8.1 mutations per megabase (Mb) and median of 8.4 per Mb. That rate is higher than rates observed in other TCGA projects including acute myelogenous leukaemia (0.56 per Mb), breast carcinoma (1.0 per Mb), ovarian cancer¹⁷ (2.1 per Mb), glioblastoma multiforme¹⁸ (2.3 per Mb) and colorectal carcinoma (3.2 per Mb) (data as of 1 February 2012, $P < 2.2 \times 10^{-16}$ by *t*-test or Wilcoxon's rank sum test for lung SQCC versus all others). In lung SQCC, CpG transitions and transversions were the most commonly observed mutation types, with mean rates of 9.9 and 10.7 per sequenced megabase of CpG context, respectively, for a total mutation rate of 20.6 per Mb. At non-CpG sites, transversions at C:G sites were more common than transitions (7.3 versus 2.9 per Mb; total = 10.2 per Mb) and more common than transversions or transitions at A:T sites (1.5 versus 1.3 per Mb; total = 2.8 per Mb).

Significantly mutated genes were identified using a modified version of the MutSig algorithm (Supplementary Methods, section 3)^{22,23}. We identified 10 genes with a false discovery rate (FDR) Q value < 0.1 (Supplementary Table 3.1): *TP53*, *CDKN2A*, *PTEN*, *PIK3CA*, *KEAP1*, *MLL2*, *HLA-A*, *NFE2L2*, *NOTCH1* and *RB1*, all of which demonstrated robust evidence of gene expression as defined by reads per kilobase of exon model per million mapped reads (RPKM) > 1 (Fig. 1). *TP53* mutation was observed in 81% of samples by automated analysis; visual review of sequencing reads identified a further 9% of samples with potential mutations in regions of sub-optimal coverage or in samples with low purity. Most observed mutations in *NOTCH1* (8 out of 17) were truncating alterations, suggesting loss-of-function, as has recently been reported for head and neck SQCCs^{22,24}. Mutations in *HLA-A* were also almost exclusively nonsense or splice site events (7 out of 8).

To increase our statistical power to detect mutated genes in the setting of the observed high background mutation rate, we performed a secondary MutSig analysis only considering genes previously observed to be mutated in cancer according to the COSMIC database.

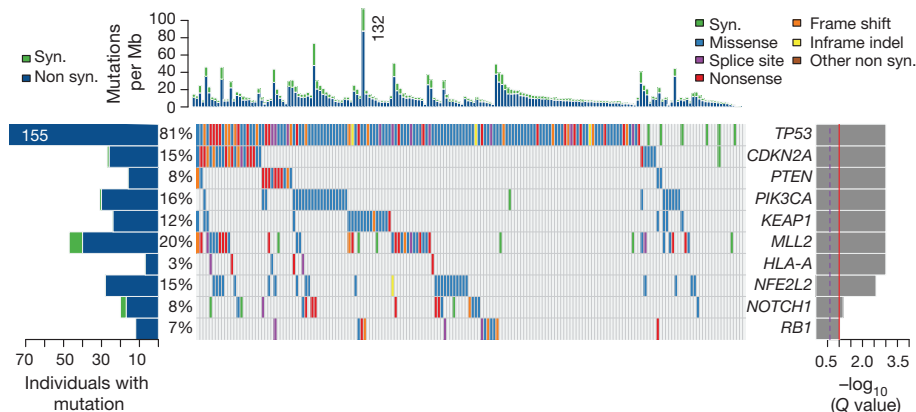


Figure 1 | Significantly mutated genes in lung SQCC. Significantly mutated genes (Q value < 0.1) identified by exome sequencing are listed vertically by Q value. The percentage of lung SQCC samples with a mutation detected by automated calling is noted at the left. Samples displayed as columns, with the overall number of mutations plotted at the top, and samples are arranged to emphasize mutual exclusivity among mutations. Syn., synonymous.

This yielded 12 other genes with $FDR < 0.1$: *FAM123B* (also known as *WTX*), *HRAS*, *FBXW7*, *SMARCA4*, *NF1*, *SMAD4*, *EGFR*, *APC*, *TSC1*, *BRAF*, *TNFAIP3* and *CREBBP* (Supplementary Table 3.1). Both the spectrum and the frequency of *EGFR* mutations differed from those seen in lung adenocarcinomas. The two most common alterations in lung adenocarcinoma, Leu858Arg and inframe deletions in exon 19, were absent, whereas two Leu861Gln mutations were detected in *EGFR*.

As described in Supplementary Fig. 3.1, we verified somatic mutations by performing an independent hybrid-recapture of 76 genes in all samples. A total of 1,289 mutations were assayed, and we achieved satisfactory coverage to have power to verify at 1,283 positions. We validated 1,235 mutations (96.2%) (Supplementary Fig. 3.1 and Supplementary Methods, section 3). We also verified mutation calls using WGS and RNA sequencing data with similar results (Supplementary Figs 3.1, 4.3 and Supplementary Methods, sections 3 and 4).

WGS was performed for 19 tumour/normal pairs with a mean computed coverage of 54 \times . A mean of 165 somatic rearrangements was found per lung SQCC tumour pair (Supplementary Fig. 3.2), a value in excess of that reported for WGS studies of other tumour types including colorectal carcinoma (75)²⁵, prostate carcinoma (108)²⁶, multiple myeloma (21)²³ and breast cancer (90)²⁷. Although most inframe coding fusions detected in WGS were validated by RNA sequencing, no recurrent rearrangements predicted to generate fusion proteins were identified (Supplementary Data 3.1 and 4.1).

Somatically altered pathways

Many of the somatic alterations we have identified in lung SQCCs seem to be drivers of pathways important to the initiation or progression of the cancer. Specifically, genes involved in the oxidative stress response and squamous differentiation were frequently altered by mutation or SCNA. We observed mutations and copy number alterations of *NFE2L2* and *KEAP1* and/or deletion or mutation of *CUL3* in 34% of cases (Fig. 2). *NFE2L2* and *KEAP1* code for proteins that bind to each other, have been shown to regulate the cell response to oxidative damage, chemo- and radiotherapy, and are somatically altered in a variety of cancer types^{28,29}. We found mutations in *NFE2L2* almost exclusively in one of two *KEAP1* interaction motifs, DLG or ETGE. Mutations in *KEAP1* and *CUL3* showed a pattern consistent with loss-of-function and were mutually exclusive with mutations in *NFE2L2* (Figs 1c and 2). PARADIGM SHIFT³⁰ analysis predicts that mutations in *NFE2L2* and *KEAP1* exert a considerable functional effect (Supplementary Fig. 7.C.1, 7.C.2 and Supplementary Methods, section 7).

We also found alterations in genes with known roles in squamous cell differentiation in 44% of samples, including overexpression and amplification of *SOX2* and *TP63*, loss-of-function mutations in *NOTCH1*, *NOTCH2* and *ASCL4* and focal deletions in *FOXP1* (Fig. 2). Although *NOTCH1* has been well characterized as an oncogene in haematological cancers³¹, *NOTCH1* and *NOTCH2* truncating mutations have been reported in cutaneous SQCCs and lung SQCCs³². Truncating mutations in *ASCL4* are the first to be reported in human

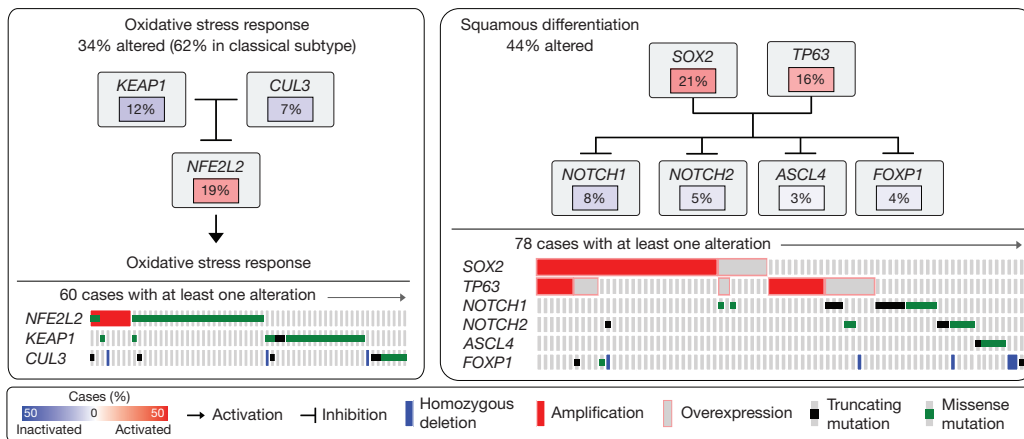


Figure 2 | Somatically altered pathways in squamous cell lung cancer. Left, alterations in oxidative stress response pathway genes as defined by somatic mutation, copy number alteration or up- or downregulation. Frequencies of alteration are expressed as a percentage of all cases, with background in red for activated genes and blue for inactivated genes. Right, alterations in genes that regulate squamous differentiation, as defined in the left panel.

cancer and may have a lineage role given the requirement for *ASCL1* for survival of small-cell lung cancer cells³³. Alterations in *NOTCH1*, *NOTCH2* and *ASCL4* were mutually exclusive and exhibited minimal overlap with amplification of *TP63* and/or *SOX2* (Fig. 2), suggesting that aberrations in those modulators of squamous cell differentiation have overlapping functional consequences.

mRNA expression profiling and subtype classification

Whole-transcriptome expression profiles were generated by RNA sequencing for the entire cohort and by microarrays for a 121-sample subset. Of 20,502 genes analysed, the mean RNA coverage indices were 19× and 6,420 RPKM (Supplementary Fig. 4.1 and Supplementary Methods, section 4). Previously reported lung SQCC gene expression-subtype signatures³⁴ were applied to both of the expression platforms, yielding four subtypes designated as classical (36%), basal (25%), secretory (24%) and primitive (15%). The concordance of subtypes between the two platforms was high (94% agreement) (Supplementary Fig. 4.2). Considerable correlations were found between the expression subtypes and genomic alterations in copy number, mutation and methylation (Fig. 3). The classical subtype was characterized by alterations in *KEAP1*, *NFE2L2* and *PTEN*, as well as pronounced hypermethylation and chromosomal instability. The 3q26 amplicon was present in all of the subtypes, but it was most characteristic of the classical subtype, which also showed the greatest overexpression of three known oncogenes on 3q: *SOX2*, *TP63* and *PIK3CA*. RNA sequencing data suggested that high expression levels of *TP63*, in samples with and without amplification of *TP63*, were associated with dominant expression of the deltaN isoform (also called p40), which lacks the amino-terminal transactivation domain, compared with the longer isoform, called tap63 (89% of tumours overexpressed deltaN compared with

tap63; $P < 2.2 \times 10^{-16}$). The short deltaN isoform is thought to function as an oncogene^{35,36}, and its expression was most enriched in the classical subtype. By contrast, the primitive expression subtype more commonly exhibited *RB1* and *PTEN* alterations, and the basal expression subtype showed *NF1* alterations (Fig. 3). Amplification of *FGFR1* and *WHSC1L1* was anticorrelated with the classical subtype and specifically with *NFE2L2* or *KEAP1* mutated samples. Although *CDKN2A* alterations are common in lung SQCCs, they are not associated with any particular expression subtype (Fig. 3).

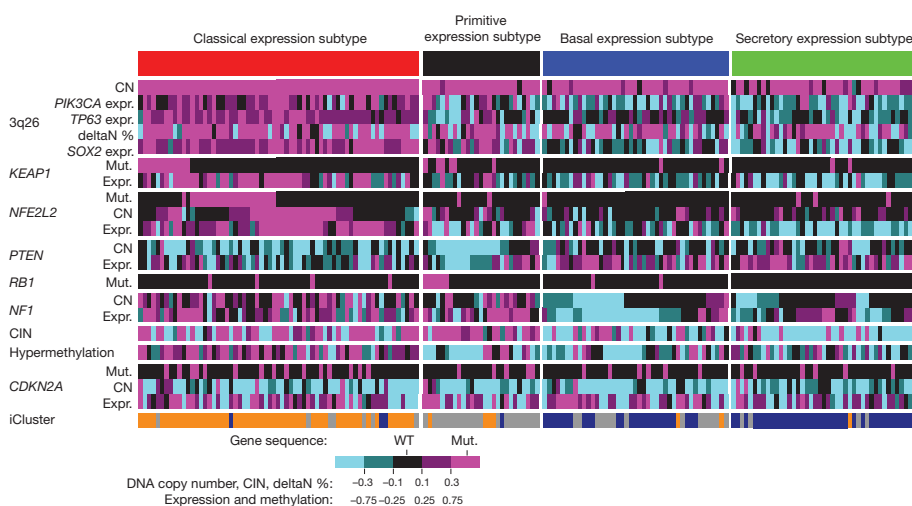
Independent clustering of miRNA and methylation data indicated association with expression subtypes. The highest overall methylation was seen in the classical subtype (Fig. 3, Supplementary Figs 5.1 and 6.1, Supplementary Methods, sections 5 and 6, Supplementary Data 6.1 and 6.2 and Supplementary Table 5.1). Integrative clustering (iCluster)³⁷ of mRNA, miRNA, methylation, SCNA and mutation data demonstrated concordance with the mRNA expression subtypes and associated alterations (Fig. 3, Supplementary Fig. 7.A.1 and Supplementary Methods, section 7). Independent correlation of somatic mutations, copy number alterations and gene expression signatures revealed notable subtype associations with alterations in the *TP53*, *PI3K*, *RB1* and *NFE2L2/KEAP1* pathways (Supplementary Fig. 7.B.1 and Supplementary Methods, section 7).

Analysis of the CDKN2A locus

Integrated multiplatform analyses showed that *CDKN2A*, a known tumour suppressor gene in lung SQCC³⁸ that encodes the p16^{INK4A} and p14^{ARF} proteins, is inactivated in 72% of cases of lung SQCC (Fig. 4a and Supplementary Data 7.1)—by epigenetic silencing by methylation (21%), inactivating mutation (18%), exon 1β skipping (4%) and homozygous deletion (29%).

Figure 3 | Gene expression subtypes integrated with genomic alterations. Tumours are displayed as columns, grouped by gene expression subtype.

Subtypes were compared by Kruskal–Wallis tests for continuous features and by Fisher’s exact tests for categorical features. Displayed features showed significant association with gene expression subtype ($P < 0.05$), except for *CDKN2A* alterations. deltaN percentage represents transcript isoform usage between the *TP63* isoforms, deltaN and tap63, as determined by RNA sequencing. Chromosomal instability (CIN) is defined by the mean of the absolute values of chromosome arm copy numbers (CN) from the GISTIC^{23,24} output. Absolute values are used so that amplification and deletion alterations are counted equally. Hypermethylation scores and iCluster assignments are described in Supplementary Figs 6.1 and 7.A.1, respectively. CIN, methylation, gene expression and deltaN values were standardized for display using z-score transformation. Expr., expression; mut., mutation; WT, wild type.



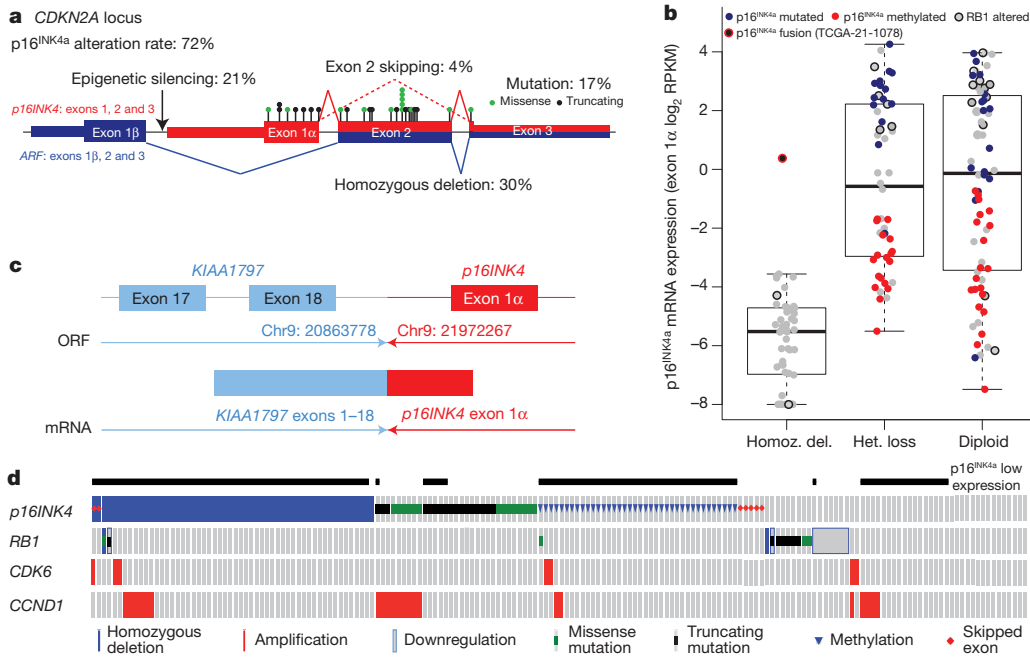


Figure 4 | Multi-faceted characterization of mechanisms of *CDKN2A* loss. **a**, Schematic view of the exon structure of *CDKN2A* demonstrating the types of alterations identified in the study. The locations of point mutation are denoted by black and green circles. **b**, *CDKN2A* expression (y axis) versus *CDKN2A* copy number (x axis). Samples are represented by circles and colour-coded by specific type of *CDKN2A* alteration. Del., deletion; het., heterozygous; homoz., homozygous. **c**, Diagram of the *KIAA1797-p16INK4* fusion identified by WGS. ORF, open reading frame. **d**, *CDKN2A* alterations and expression levels (binary) in each sample.

Analysis of mRNA expression across the *CDKN2A* locus revealed four distinct patterns of expression: complete absence of both *p16INK4* and *ARF* (33%); expression of high levels of both *p16INK4* and *ARF* (31%); high expression of *ARF* and absence of *p16INK4* (31%); or expression of a transcript that represents a splicing of exon 1 β from *ARF* with the shared exon 3 of *ARF* and *p16INK4*, generating a premature stop codon (4%) (Supplementary Fig. 4.4). Almost all of the cases completely lacking *p16INK4* and *ARF* expression showed homozygous deletion (Fig. 4b and Supplementary Data 7.1). In one case, *p16INK4* expression was detected but analysis of WGS data demonstrated an intergenic fusion event that resulted in detectable transcription between exon 1 α *p16INK4* and exon 18 of *KIAA1797* (Fig. 4b, c). Interestingly, combined analysis of WGS and RNA sequencing data identified tumour suppressor gene inactivation by intra- or interchromosomal rearrangement in *P TEN*, *NOTCH1*, *ARID1A*, *CTNNA2*, *VHL* and *NF1*, in eight further cases (Supplementary Data 3.1 and 4.1).

In addition to homozygous deletion, there are frequent mutational events in *CDKN2A* (Fig. 4b and Supplementary Data 7.1). These account for 45% of the 56 cases with high *p16INK4* and *ARF* expression. Furthermore, methylation of the exon 1 α promoter accounts for many other cases of *CDKN2A* inactivation (70% of lung SQCCs with *ARF* expression in the absence of detectable *p16INK4*). Seven other tumours in the high-*ARF*/low-*INK4A* group had documented mutations of *INK4A*, primarily nonsense mutations, suggesting nonsense-mediated decay as a mechanism. Of the 28% of tumours without *CDKN2A* alterations, *RB1* mutations were identified in eight cases and *CDK6* amplification in one case (Fig. 4d).

Therapeutic targets

Molecularly targeted agents are now commonly used in patients with adenocarcinoma of the lung, whereas no effective targeted agents have been developed specifically for lung SQCCs¹³. We analysed our genomic data for evidence of the two common genomic alterations in adenocarcinomas of the lung: *EGFR* and *KRAS* mutations. Only one sample had a *KRAS* codon 61 mutation, and there were no exon 19 deletions or Leu858Arg mutations in *EGFR*. However, amplifications of *EGFR* were found in 7% of cases, as were two instances of the Leu861Gln *EGFR* mutation, which confers sensitivity to erlotinib and gefitinib³⁹.

The presence of new potential therapeutic targets in lung SQCC was suggested by the observation that 96% (171 out of 178) of tumours

contain one or more mutations in tyrosine kinases, serine/threonine kinases, phosphatidylinositol-3-OH kinase (PI(3)K) catalytic and regulatory subunits, nuclear hormone receptors, G-protein-coupled receptors, proteases and tyrosine phosphatases (Supplementary Fig. 7.D.1a and Supplementary Data 7.2 and 7.3). From 50 to 77% of the mutations were predicted to have a medium or high functional effect as determined by the mutation assessor score⁴⁰ (Supplementary Fig. 7.D.1a), and 39% of tyrosine and 42% of serine/threonine kinase mutations were located in the kinase domain. Many of the alterations were in known oncogenes and tumour suppressors, as defined in the COSMIC database (Supplementary Data 7.3).

We selected potential therapeutic targets based on several features, including (1) availability of a US Food and Drug Administration (FDA)-approved targeted therapeutic agent or one under study in current clinical trials (Supplementary Data 7.2); (2) confirmation of the altered allele in RNA sequencing; and (3) the mutation assessor score⁴⁰. Using those criteria, we identified 114 cases with somatic alteration of a potentially targetable gene (64%) (Supplementary Fig. 7.D.1b and Supplementary Data 7.4). Among these, we identified three families of tyrosine kinases, the erythroblastic leukaemia viral oncogene homologues (ERBBs), fibroblast growth factor receptors (FGFRs) and Janus kinases (JAKs), all of which were found to be mutated and/or amplified⁴¹. As discussed for *EGFR*, the mutational spectra in these potential therapeutic targets differed from those in lung adenocarcinoma (Supplementary Fig. 7.D.2)⁴².

To complement a gene-centred search for potential therapeutic targets, we analysed core cellular pathways known to represent potential therapeutic vulnerabilities: PI(3)K/AKT, receptor tyrosine kinase (RTK) and RAS. Analysis of the 178 lung SQCCs revealed alteration in at least one of those pathways in 69% of samples after restriction of the analysis to mutations confirmed by RNA sequencing and to amplifications associated with overexpression of the target gene (Fig. 5). Mutational events that have been curated in COSMIC are also shown in Supplementary Fig. 7D.2, as is the distribution of mutations, amplifications and overexpression of the genes depicted in Fig. 5. (A summary of all samples and their significant mutations and copy number alterations, including alterations in Fig. 5, is shown in Supplementary Data 7.5.) Specifically, one of the components of the PI(3)K/AKT pathway was altered in 47% of tumours and RTK signalling probably affected by events such as *EGFR* amplification, *BRAF* mutation or *FGFR* amplification or mutation in 26% of tumours

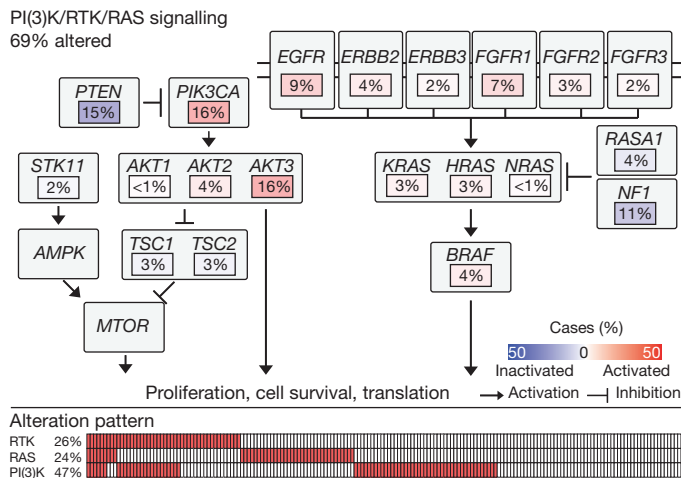


Figure 5 | Alterations in targetable oncogenic pathways in lung SQCCs. Pathway diagram showing the percentage of samples with alterations in the PI(3)K/RTK/RAS pathways. Alterations are defined by somatic mutations, homozygous deletions, high-level, focal amplifications, and, in some cases, by significant up- or downregulation of gene expression (*AKT3*, *FGFR1*, *PTEN*).

(Fig. 5 and Supplementary Fig. 7.D.3). Alterations in the PI(3)K/AKT pathway genes were mutually exclusive with *EGFR* alterations as identified by MEMo⁴³ (Supplementary Fig. 7.D.4). Although the dependence of lung SQCC on many of these individual alterations remains to be defined functionally, this analysis suggests new areas for potential therapeutic development in this cancer.

Discussion

Lung SQCCs are characterized by a high overall mutation rate of 8.1 mutations per megabase and marked genomic complexity. Similar to high-grade serous ovarian carcinoma¹⁷, almost all lung SQCCs display somatic mutation of *TP53*. There were also frequent alterations in the following pathways: *CDKN2A/RB1*, *NFE2L2/KEAP1/CUL3*, *PI3K/AKT* and *SOX2/TP63/NOTCH1* pathways, providing evidence of common dysfunction in cell cycle control, response to oxidative stress, apoptotic signalling and/or squamous cell differentiation. Pathway alterations clustered according to expression-subtype in many cases, suggesting that those subtypes have a biological basis.

A role for somatic mutation in the cancer hallmark of avoiding immune destruction⁴⁴ is suggested by the presence of inactivating mutations in the *HLA-A* gene. Somatic loss-of-function alterations of *HLA-A* have not been reported previously in genomic studies of lung cancer. Given the recently reported efficacy of anti-programmed death 1 (PD1)⁴⁵ and anti-cytotoxic T-lymphocyte antigen 4 (CTLA4) antibodies in non-small-cell lung cancer⁴⁶, these *HLA-A* mutations suggest a possible role for genotypic selection of patients for immunotherapies.

Targeted kinase inhibitors have been successfully used for the treatment of lung adenocarcinoma but minimally so in lung SQCC. The observations reported here suggest that a detailed understanding of the possible targets in lung SQCCs may identify targeted therapeutic approaches. Whereas *EGFR* and *KRAS* mutations, the two most common oncogenic aberrations in lung adenocarcinoma, are extremely rare in lung SQCC, alterations in the FGFR kinase family are common. Lung SQCCs also share many alterations in common with head and neck squamous cell carcinomas without evidence of human papilloma virus infection, including mutation in *PIK3CA*, *PTEN*, *TP53*, *CDKN2A*, *NOTCH1* and *HRAS*^{22,24}, suggesting that the biology of these two diseases may be similar.

The current study has identified a potentially targetable gene or pathway alteration in most lung SQCC samples studied. The data presented here can help to organize efforts to analyse lung SQCC clinical tumour specimens for a panel of specific, actionable mutations to select patients for appropriately targeted clinical trials. These

data could thereby help to facilitate effective personalized therapy for this deadly disease.

METHODS SUMMARY

All specimens were obtained from patients with appropriate consent from the relevant Institutional Review Board. DNA and RNA were collected from samples using the Allprep kit (Qiagen). We used commercial technology for capture and sequencing of exomes from tumour DNA and normal DNA and whole-genome shotgun sequencing. Significantly mutated genes were identified by comparing them with expectation models based on the exact measured rates of specific sequence lesions. GISTIC^{23,24} analysis of the circular-binary-segmented Affymetrix SNP 6.0 copy number data was used to identify recurrent amplification and deletion peaks. Consensus clustering approaches were used to analyse mRNA, miRNA and methylation subtypes using previous approaches^{20,21,34,38,41,44}.

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Author Information The primary and processed data used to generate the analyses presented here can be downloaded by registered users from The Cancer Genome Atlas (<https://tcga-data.nci.nih.gov/tcga/tcgaDownload.jsp>, <https://cghub.ucsc.edu/> and https://tcga-data.nci.nih.gov/docs/publications/lusc_2012/). Reprints and permissions information is available at www.nature.com/reprints. This paper is distributed under the terms of the Creative Commons Attribution-Non-Commercial-Share Alike licence, and the online version of the paper is freely available to all readers. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M. Meyerson (matthew_meyerson@dfci.harvard.edu).

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CORRIGENDUM

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In this Article, author Kristen Rodgers was spelt incorrectly. This error has been corrected in the HTML and PDF of the original paper.