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Review

Genetic alterations defining NSCLC subtypes and their therapeutic implications

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ABSTRACT

Lung cancer is the leading cause of cancer death worldwide, accounting for more deaths than breast, prostate and colon cancer combined. While treatment decisions are determined primarily by stage, therapeutically non small cell lung cancer (NSCLC) has traditionally been treated as a single disease. However, recent findings have led to the recognition of histology and molecular subtypes as important determinants in treatment selection. Identifying the genetic differences that define these molecular and histological subtypes has the potential to impact treatment and as such is currently the focus of much research. Microarray and genomic sequencing efforts have provided unparalleled insight into the genomes of lung cancer subtypes, specifically adenocarcinoma (AC) and squamous cell carcinoma (SqCC), revealing subtype specific genomic alterations and molecular subtypes as well as differences in cell signaling pathways. In this review, we discuss the recurrent genomic alterations characteristic of AC and SqCC (including molecular subtypes), their therapeutic implications and emerging clinical practices aimed at tailoring treatments based on a tumor's molecular alterations with the hope of improving patient response and survival.

1. Introduction

Lung cancer is the leading cause of cancer death in the world, with an estimated 251,760 new cases and 180,440 deaths in Canada and the U.S. in 2012 [1,2]. Despite recent advances in the field, the 5-year survival rate has failed to improve significantly over the last 30 years, and remains a meager 15%, largely due to limitations in detection and treatment strategies [3]. Histologically, lung cancer is classified into two broad categories; small-cell lung cancer (SCLC), occurring in approximately 15% of patients and the more prevalent NSCLC, which accounts for approximately 85% of cases [4]. NSCLC can be further divided into 3 major histological subtypes: adenocarcinoma (AC), squamous cell carcinoma (SqCC) and large cell carcinoma, with AC and SqCC accounting for over 70% of NSCLC cases [4]. Despite sharing many biological features, subtypes differ in their cell of origin, location within the lung, and growth pattern, suggesting they are distinct diseases that develop through differential molecular mechanisms.

Until recently, NSCLC was treated as a single disease with a "one size fits all" therapeutic approach due to the similar therapeutic effects of conventional chemotherapeutic agents. However, with the observation that subtypes display distinct patterns of genomic alterations and evidence from clinical trials demonstrating that tumor histology influences response rates, toxicity and progression free survival of targeted drugs such as bevacizumab, pemetrexed and epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI's), histology is now recognized as an important factor in treatment selection. The development of targeted therapies, specifically TKIs, which act as competitive inhibitors of the ATP binding pocket, blocking downstream signaling have provided improvements in therapeutic response and highlight the clinical benefit of identifying and targeting biologically relevant alterations [5,6]. As a result of the success of *EGFR* TKIs, and the profound clinical benefit of targeted therapies in other cancers including breast and chronic myeloid leukemia, a number of targeted therapies against other recurrent molecular alterations in NSCLC are currently in development, and molecular classification of tumors is becoming increasingly important in treatment selection.

As the landscapes of genomic alterations and associated clinical features have been extensively characterized in AC and SqCC, this review will focus explicitly on the two most predominant subtypes of NSCLC. This article summarizes the spectrum of shared

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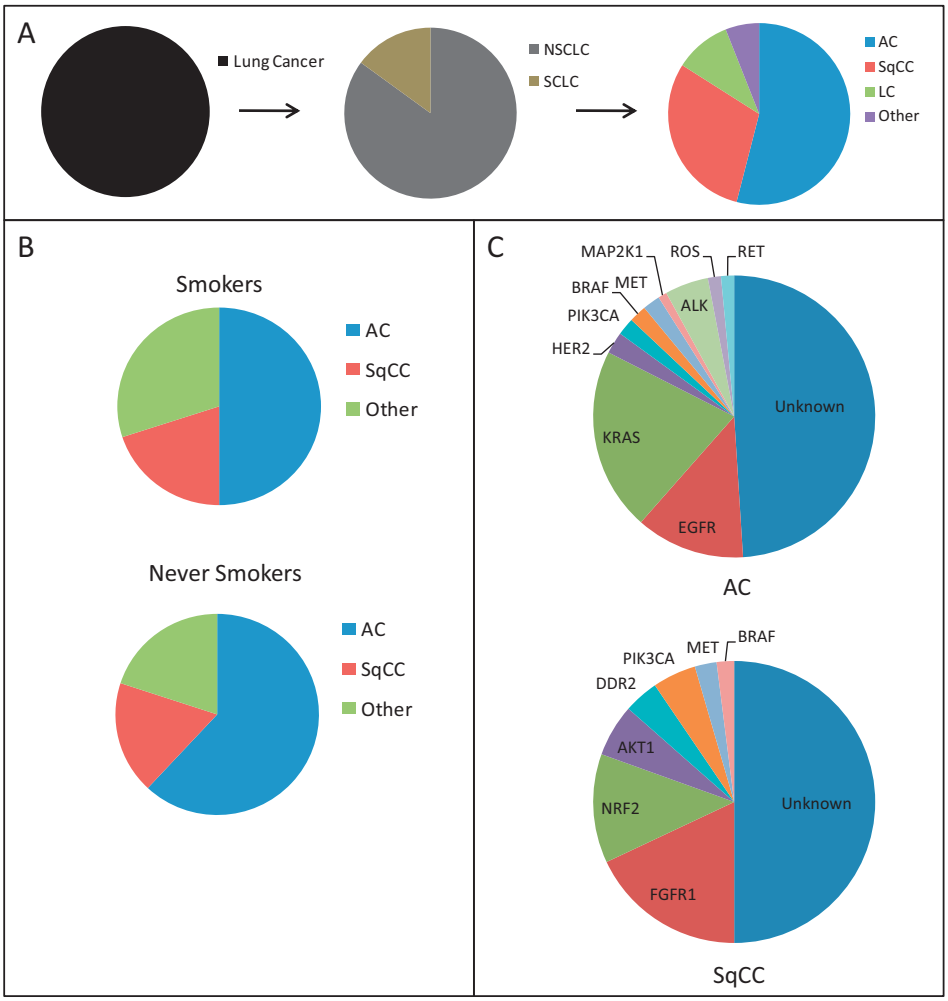

Fig. 1. Lung cancer subtypes. (A) Evolution of histological subtyping of lung cancer. The designation other encompasses minor histologies which includes NSCLC NOS, adenosquamous and sarcomatoid. (B) Composition of histological subtypes in lung cancer of smokers and never smokers. (C) Molecular subtypes of AC and SqCC. Only hyperactive oncogenes that are mutually exclusive to one another and therapeutically targetable are displayed. Pie charts depict the proportions of the patient population with a given histological or molecular subtype.

and unique genetic alterations characteristic of AC and SqCC, from gene expression signatures and patterns of DNA methylation and copy number alterations to mutations and chromosomal rearrangements identified by genome sequencing. The therapeutic implications of 'actionable' alterations and emerging practices aimed at creating a personalized approach to the treatment of lung cancer and improving survival are also addressed.

2. Histopathology of NSCLC

While all histological subtypes of lung cancer are associated with cigarette smoking, SqCC and SCLC (Fig. 1A), both of which arise predominantly in the central airways are most strongly associated with a history of smoking. Within the last few decades, there has been a dramatic shift in the global trends of lung cancer histology, with a steady decline in SCLC and SqCC such that AC is now the most common subtype of lung cancer (Fig. 1B). These changes are largely believed to be due to widespread changes in cigarette composition (lower tar and nicotine content) which has led to a change in smoking behavior with smokers smoking more frequently and inhaling deeper in an attempt to achieve the same effect, causing tobacco carcinogens to be deposited further into the lung periphery. AC, now accounts for roughly half of all lung cancer cases and typically arises in the glandular epithelium of the lung parenchyma from type II pneumocytes or clara cells whereas SqCC, which accounts for ~30% of lung cancer and originates from basal cells in the central airways [7] (Fig. 1A). Large cell carcinomas (LCC), are a diverse group of poorly or undifferentiated tumors with poor prognosis that can have neuroendocrine features and can harbor components or AC, SqCC or SCLC. In addition to these three main subtypes, there exists a small subset of tumors with mixed, (sarcomatoid and adenosquamous carcinomas) or not otherwise specified (NOS) histologies and clinical characteristics that are indistinct from other subtypes. Due to the therapeutic importance of distinguishing histological subtypes, in 2011 the IASLC/ATS/ERS proposed new guidelines for the pathological classification of NOS tumors [7]. The application of immunohistochemical panels containing a mixture of AC and SqCC markers and EGFR and ALK mutation testing have refined NSCLC classification, significantly reducing the percent of NOS tumors diagnosed [8,9]. The inclusion of additional molecular alterations with evidence supporting a subtype specific pattern of alteration (ex: *FGFR1* amplification and *DDR2* mutation in SqCC) as well as molecular profiling of less characterized subtypes such as LCC will provide insight into the biology of these tumors and potentially identify novel genetic alterations that could aid in further refining pathological diagnosis and classification of NSCLC subtypes.

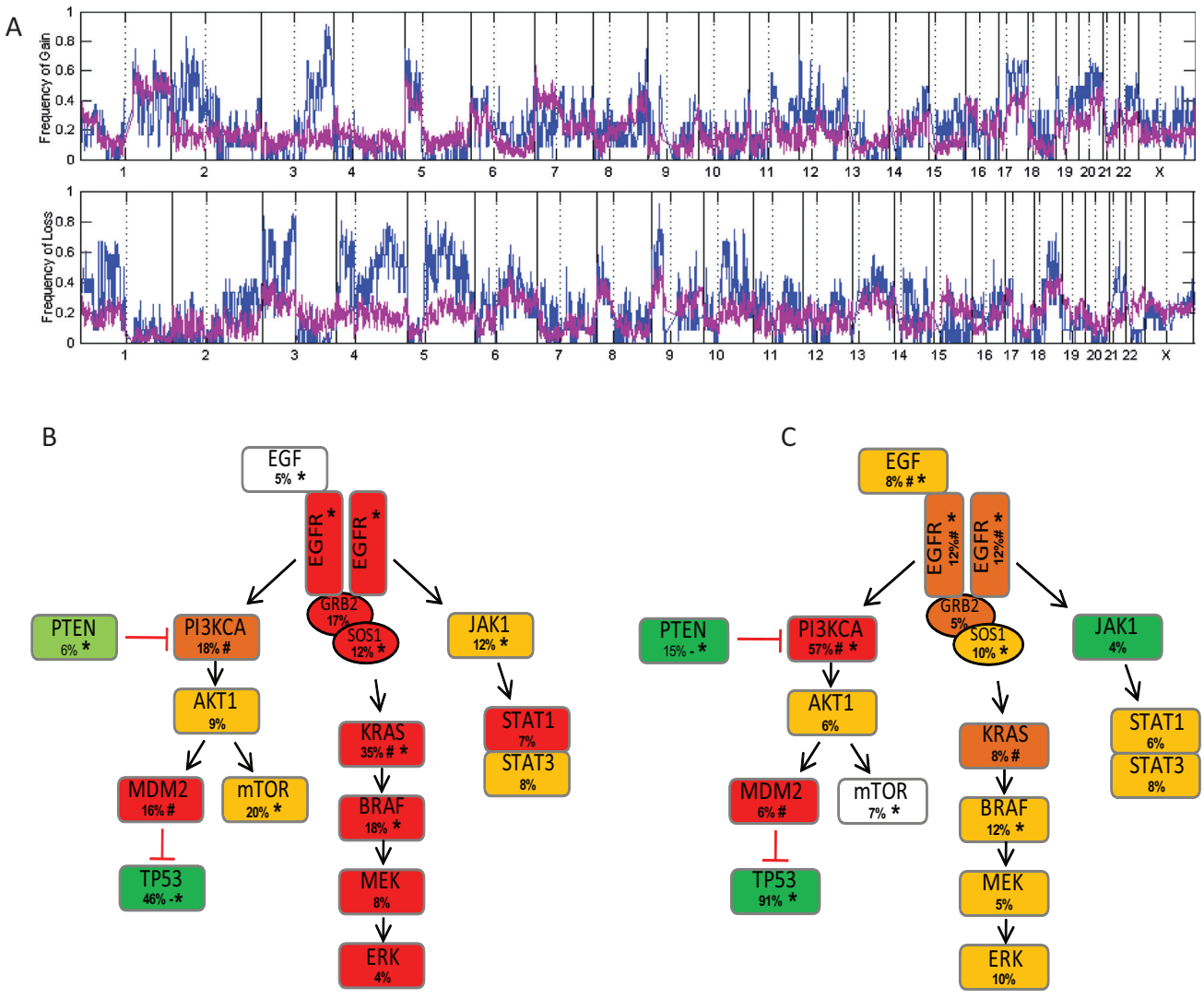

Fig. 2. Differential patterns of alteration in AC and SqCC. (A) Frequency plot of copy number alterations across the entire human genome for 83AC (purple) and 12 SqCC (blue) relative to matched non-malignant tissue. Solid vertical black lines indicate chromosome boundaries and dotted lines chromosome arm boundaries. The frequency of copy gain is depicted in the top panel, and copy loss in the bottom. The greatest regions of disparity include gain of 3q, loss of 3p, chromosome 4 and 5q in SqCC, and gain of 7p in AC. Simplified EGFR signaling pathway diagram depicting the frequency of disruption, prominent genomic mechanisms of alteration and corresponding expression in AC (B) and SqCC (C) using data from cbio portal. The color of the gene depicts the trend in expression; red: overexpression (OE), orange: predominant OE, but some underexpression (UE), yellow: both OE and UE observed at a fairly equal frequency, light green: predominant UE but some OE, dark green: UE and empty: no change in expression. The frequency of alteration is displayed below the gene name and the mechanisms of alteration are denoted by #, - and * for amplification, homozygous deletion and mutation respectively. Within the EGFR pathway, the genes with the most different alteration status between subtypes include PIK3CA, JAK1 and KRAS. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3. Molecular features associated with cell lineage and smoking

Tumor biology is highly influenced by the lineage and differentiation state of tumor precursor cells, and lineage survival pathways required for normal development are often aberrantly activated as a result of genomic alterations promoting the continued survival and proliferation of cancer cells [10]. Two such lineage survival oncogenes have been identified in NSCLC; *NKX2-1/TITF1* in AC [11] and *SOX2* in SqCC [12]. *NKX2-1* and SOX2 are transcription factors that play essential roles in lung development and the correct differentiation of respiratory cell types [13–15]. Clinically, NKX2-1 along with CK7, mucin, Napsin A p63, p40 and CK5/6 are used as immunohistochemical markers for histological subtyping [16–18]. Although SOX2 is not frequently used as an IHC marker, high expression is associated with poorly differentiated tumors, which typically have a poorer prognosis [19]. In addition to these two lineage specific oncogenes, Lockwood et al., identified a squamous specific oncogene, *BRF2*, located in a chromosome region of frequent amplification in SqCC (Fig. 2A). Activation of *BRF2* plays a key role in SqCC tumorigenesis via an increase in Pol III mediated transcription and is frequently altered in pre-neoplastic lesions, suggesting it is an early event in SqCC development and a potential lineage specific oncogene [20].

In addition to the histological differences, cigarette smoking is associated with specific clinical and genetic features. Never-smoker lung cancer, which accounts for up to 25% of all lung cancers worldwide [21] are more strongly associated with East Asian ethnicity, female gender and AC histology. Genetically, never smokers are associated with a higher prevalence of *EGFR*, *PTEN*, *ALK*, *ROS1*, and *RET* alterations, whereas *KRAS*, *TP53*, *BRAF*, *STK11, and JAK2/3* mutations and hypermethylation of *p16* and *LGALS4* are more common in smokers [22–25]. More recently smoking dependent differences have been shown to extend beyond specific gene alterations, to

differential patterns of chromosomal aberrations and differences in the proportion of tumor genomes affected by segmental genomic alterations [26], lower mutational frequencies and higher rates of transitions verse transversions in never smokers compared to smokers [22,23]. Collectively, these findings support the notion that diverse genetic mechanisms underlie the development of lung tumors in smokers and never smokers within a single histological subtype, indicating smoking status is an important clinical variable that should be considered when comparing AC and SqCC.

4. Alterations of genes and pathways characterizing AC and SqCC

The histological differences and disparate clinical behaviors of AC and SqCC suggest distinct molecular mechanisms underlie these phenotypic differences. Subtype specific patterns of genomic alterations have been observed across all 'omics levels, however how key genes and pathways interact and are differentially disrupted between subtypes, which can have important therapeutic implications, has only recently begun to be assessed.

4.1. Gene expression

Gene expression signatures have shown the ability to define and distinguish histological subtypes, [27–31] morphological subtypes within AC [27,28] and SqCC [32] as well as distinguish tumor from non-malignant tissue [33–36], yet their clinical utility is limited due to the lack of overlap between subtype signatures. Interestingly, functional overlap between subtype specific signatures has been observed, suggesting disruption of specific pathways is selected for rather than specific genes. Deregulation of antioxidant proteins, detoxification genes and overexpression of cytokeratins and cytokeratin-regulatory genes (*GSTT1*, *CEL*, and *PRDX6*) often characterize SqCC tumors [27–31], whereas disruption of surfactant-related and small airway-associated genes (*SFTPA2, SFTPB, MUC1, and NAPSA*) are typically altered in AC [27–30,37,38]. These functions are largely associated with the histological properties of the cells or origin from which these subtypes develop, further highlighting the contribution of histology to tumorigenesis.

4.2. Copy number alterations

DNA copy number alterations (CNAs) are a prominent mechanism of gene disruption in NSCLC [11,39–48]. Although very few CNAs are altered exclusively in a single subtype, many regions are altered at significantly different frequencies between subtypes and therefore deemed regions of subtype specific CNA (Fig. 2A and Table 1) [40,41,43]. For example, a recent analysis of over 2000 tumors identified 13 subtype-specific regions with at least a 25% difference in the frequency of alteration between subtypes [49]. Amidst all copy number studies, the most prominent and consistent difference between subtypes is amplification of 3q in SqCC (Fig. 2A) [12,39,40,42,44,46,48,50].

4.3. Whole genome sequencing

Advances in exome and whole genome sequencing technologies have enabled high throughput identification of mutations, copy number aberrations, and structural alterations such as gene fusions and chromosomal rearrangements in a genome-wide, unbiased manner. One of the first high throughput sequencing studies of lung cancer interrogated 623 cancer related genes in 188 AC samples and identified over 1000 somatic mutations and 26 frequently mutated genes. These included genes known to be frequently mutated in lung cancer such as *TP53*, *BRAF*, *ERBB2*, *KRAS*, *STK11*, *EGFR*, *PIK3CA*, *PTEN* and *CDKNA*, in addition to *NF1*, *RB1*, *ATM*, *FGFR4*, and *ERBB4* which had no previous evidence of recurrent mutation in lung cancer [51]. Since then, sequencing of AC and matched non malignant tissue has continued to identify novel mutations and gene fusions (including *ARID1A*, *SMARA4*, *ASH1L*, *U2AF1* and *KIF5B-RET*) while simultaneously revealing immense mutational heterogeneity both within (intra) and between (inter) patients [23,52–54]. For example, a single AC tumor was found to have over 50,000 variants, of which 391 affected coding sequences [55]. Substantial inter- and intra-tumoral heterogeneity has also been observed in copy number and gene expression profiling of AC, suggesting the mechanisms underlying tumorigenesis in this subtype may be extremely diverse. Whether this heterogeneity could be due in part to the histological subgroups of AC, or some other feature has yet to be elucidated.

Table 1
Recurrent amplifications and deletions with known oncogenes and tumor suppressors in lung cancer.

Chromosomal region	Alteration	Subtype	Candidate oncogene/TSG
1q21.2	Amplification	AC	ARNT
3q26.3-q27	Amplification	SqCC	SOX2 and PIK3CA
4q12	Amplification	SqCC	PDGFRA
5p15.33	Amplification	AC	TERT
7p11.2	Amplification	NSCLC	EGFR
8q12	Amplification	SqCC	BRF2 and FGFR1
9p21.3	Deletion	NSCLC	CDKN2A/B
10q23.31	Deletion	NSCLC	PTEN
12q15	Amplification	AC	MDM2
14q13.33	Amplification	AC	NKX2-1
17p	Deletion	NSCLC	TP53
17q11.2	Deletion	SqCC	Nf1
17q12	Amplification	AC	ERBB2
19p	Deletion	AC	STK11/LKB1

To date, the most comprehensive sequencing analysis of SqCC was performed by the Cancer Genome Atlas (TCGA) research network. In addition to the identification of a number of frequently mutated genes; *TP53*, *CDKN2A*, *PTEN*, *PIK3CA*, *KEAP1*, *MLL2*, *HLA-A*, *NFE2L2*, *NOTCH1 and RB1*, their analysis identified 360 exonic mutations, 165 genomic rearrangements, and an average of 323 CNAs per sample [50]. While mutation patterns specific to AC and SqCC have emerged, analogous to CNA few are exclusive to a single subtype and many, *LRRC7*, *SLC7A13*, *PCDH11X*, *CSMD3*, *DNAH3*, *CD1B*, *CACNA2D1*, *KEAP1*, *PIK3C2B* and *CTNNA3* for example, occur at similar frequencies in both subtypes [56]. Interestingly, SqCC genomes were found to have a significantly higher rate of CNAs and mutations than all other tumor types (glioblastoma multiforme, ovarian, colorectal, breast and renal cell carcinoma) profiled by the TCGA thus far. High mutation rates have also been observed in AC [57], suggesting lung cancers as a whole are more genetically unstable, which could be due to the carcinogenic effects of cigarettes. Studies aimed at identifying genes driving AC and SqCC phenotypes must therefore consider the highly complex genomic backgrounds of these tumors when deciphering biologically and therapeutically relevant alterations. Taken together, these studies highlight the heterogeneity and genomic complexity of lung cancer subtypes. Expected to be released this year, the TCGA's characterization of AC will provide a similar in depth description of the spectrum of alterations in AC and allow for a comprehensive multidimensional comparison between AC and SqCC.

4.4. DNA methylation

Epigenetic marks such as DNA methylation are important regulators of somatically heritable changes in gene expression. DNA methylation is a tissue-specific and inherently reversible gene

regulatory alteration targeted for chemoprevention and treatment and as potential diagnostic and prognostic biomarkers in malignant and non-malignant tissues [58]. DNA methylation profiling of NSCLC has identified hundreds of aberrantly methylated genes [59–63]. However, to date most genome-wide epigenetic studies lack corresponding gene expression level data, which in the context of determining functional consequences of DNA methylation alterations to lung cancer biology, is limiting. In SqCC, integration of global DNA methylation and expression profiles indicate a role for aberrant DNA methylation in DNA replication, recombination and repair functions, and that methylation of *HOXA2* and *HOXA10* may have prognostic relevance [64,65]. In AC, aberrantly methylated genes are enriched for cell differentiation, cell cycle regulation, epithelial to mesenchymal transition and RAS and WNT signaling pathways [66].

4.5. Non-coding RNAs

The human genome contains approximately 20,000 protein-coding genes, representing <2% of the genome [67]. Within the past decade sequencing technologies have revealed that over 90% of the genome is actively transcribed and includes a collection of antisense and non-coding RNA (ncRNA) transcripts [68,69]. ncRNA are transcripts that lack open reading frames and do not typically encode a protein, the best studied of which are miRNA. Similar to gene expression, miRNA signatures can accurately separate histological subtypes and are thought to be as good or even superior to global mRNA expression profiles in their ability to accurately classify NSCLC subtypes [70]. *miR-205* has been shown as a highly specific marker for SqCC [71], while in AC, specific miRNAs have been shown to associate with mutation patterns. *miR-155* is upregulated exclusively in AC with wildtype *EGFR* and *KRAS*, while *miR-21* and *miR-25* are upregulated in *EGFR* mutant AC and *miR-495* is up-regulated in *KRAS* positive AC [72,73]. The study of long ncRNAs (lncRNAs) in lung cancer is still an emerging field, and to date no lncRNAs have demonstrated diagnostic or therapeutic potential in lung cancer. However, diagnostic lncRNAs have been identified in other cancer types including prostate and liver cancer [74,75] and *metastasis-associated lung adenocarcinoma transcript 1* (*MALAT1*) is known to be associated with metastasis and poor prognosis in NSCLC, highlighting its potential as a prognostic marker [76]. Based on these and other recent findings, non-coding transcripts may be just as important to tumor biology and therapeutics as protein coding transcripts, underscoring their significance.

4.6. Pathways associated with subtype specific genomic alterations

While the application of single dimensional analyses (expression, copy number, or mutation studies alone) are informative for identifying disrupted genes, they often overlook genes disrupted at low frequencies and are not capable of distinguishing causal from passenger events [77]. The integration of multiple dimensions of 'omics data provides a more comprehensive understanding of the genetic mechanisms affecting a tumor as it not only enables the identification of genes with concurrent DNA and expression alterations which are more likely to be driver alterations, but also genes disrupted by multiple mechanisms but at low frequencies by any single mechanism (Fig. 2B and C) [77]. However, gene discovery on its own provides limited information regarding tumor biology. The inclusion of pathway or network analysis (Ingenuity Pathway Analysis, Kyoto Encyclopaedia of Genes and Genomes (KEGG) and Gene Set Enrichment Analysis to name a few) can be a useful tool to provide biological context to a set of alterations and aid in interpreting how they work in conjunction to promote tumorigenesis (Fig. 2B and C). Furthermore, understanding where a pathway is disrupted can reveal novel therapeutic intervention points that may help achieve better therapeutic response.

Numerous studies mapping multiple omics dimensions to annotated pathways and cancer hallmarks support the notion that alterations work in concert to selectively deregulate pathways and further confirm that AC and SqCC develop through distinct oncogenic pathways [11,50,51,65]. Single subtype analyses have identified several affected pathways/hallmarks including; focal adhesions, cell cycle, activation of the *JAK/STAT* pathway and sustainment of proliferation in AC [22,52] and oxidative stress response, squamous differentiation and deregulation of the PI3K pathway in SqCC [51]. Of therapeutic relevance, was the finding that ~70% of SqCC tumors had alterations in one of the PI3K/AKT, receptor tyrosine kinase, or RAS pathways, however the optimal intervention points of these pathways are still under investigation. Work by our group comparing AC and SqCC identified 778 subtype-specific genes (altered by CNA or DNA methylation and a two-fold expression change) which were found to differentially disrupt cellular pathways and networks, including down-regulation of the HNF4alpha pathway in AC and disruption of histone modifying enzymes and the E2F1 transcription factor in SqCC [65]. Differential pathway activation of the cell cycle in AC, and DNA repair in SqCC, have been reported along with differences in multiple metabolic pathways [78].

4.7. Therapeutic implications

With distinct patterns of genetic disruption underscoring tumor development, it is unrealistic to assume AC and SqCC would have similar responses to all chemotherapies, especially those targeting specific proteins. Both bevacizumab, a monoclonal antibody against *vascular endothelial growth factor* (*VEGF*) and pemetrexed, an antifolate chemotherapy that targets *thymidylate synthetase* (*TS*) are contraindicated in SqCC due to an increased risk of pulmonary hemorrhage and reduced survival times, respectively [79–81]. TS gene expression has been shown to be predictive of pemetrexed efficiency [82,83] and is elevated in SqCC compared to AC, providing an explanation for the reduced efficacy in these patients [81,84]. In silico screening of compounds capable of "reversing" gene expression signatures has been shown to identify compounds with subtype specific efficacy. For example, treatment of lung cancer cell lines with the HDAC inhibitor Trichostatin A, revealed SqCC lines were significantly more sensitive than AC lines [65]. These findings highlight the potential importance of information about the underlying biology to inform decisions regarding treatment regimes, such that treatments can be tailored to the individual to potentially improve patient response and survival.

5. Targetable genetic alterations

The discovery of improved responses and outcomes with EGFR TKIs in lung cancer patients harboring *EGFR* mutations launched the search for additional actionable alterations and marked the beginning of a new era in which NSCLCs are defined by their driver alterations (Fig. 1). To date only one other targeted agent, a small molecule inhibitor of ALK (crizotinib) has been approved for clinical use, however more than a dozen other targeted therapies are currently being assessed in clinical trials. Table 2 lists the most common actionable alterations identified in NSCLC along with targeted agents developed against them and a brief description about their mechanism of action. Specific details of these inhibitors have been extensively reviewed elsewhere [85–89].

Table 2
Molecular subtypes of lung cancer.

Gene	Location	Frequency %AC	Frequency % SqCC	Alteration	Clinical features	Drug sensitivity	References
EGFR	7p12	10–15	<5	del exon 19, L858R	AC, AS, F, NS	Gefitinib* and Erlotinib*	[6,135–138]
KRAS	12p12.1	21	6	Mutation of amino acid 12 and 13	AC, F, Y, CS	None	[97,138]
MEK1	15q22.1	1	N/A	E56P, K57N, D67N	AC	Selumetinib (2), Trametinib (2)	[87,139,140]
HER2	17q12	<5	Rare	Exon 20 Insertion	AC, AS, F, NS	Neratinib (2), Afatinib (3), Trastuzumab (3)	[87]
ALK	2p23	4–6	Rare	EML4 or KIF5B fusion	AC, Y, NS	AP26113 (2), Ganetespib (2), LDK378 (2), CH5424802 (2), Sunitinib (2), Crizotinib*	[101–104,141–143]
ROS	6q22	1–2	N/A	Fusion with multiple genes	AC, Y, NS	Crizotinib (c + 1 patient)	[22,23,111,144–148]
RET	10q11.2	1–2	N/A	Fused with KIF5B, CCDC6, or NCOA4	AC, NS	Vandetanib (2), Sunitinib (c), Sorafenib (c), Cabozantinib (2), Lenvatinib (2), Ponatinib (2)	[54,88,107–111,149,150]
PDGFRA	4q12	3–7	8–12	Amplification	SqCC	Sorafenib (3), Sunitinib, Crenolanib (2), pazopanib (2), Axetinib (2)	[151,152]
FGFR1	8p12	1	16–20	Amplification	SqCC	Dovitinib (2), PD173074 (v), AZD4547 (2)	[12,153–155]
NRF2	2q31	1–2	10–15	Mutation Exon 2	SqCC	None	[50,156–159]
DDR2	1q23.3	1	4	Mutation	SqCC	Dasatinib (2), Nilotinib (c), Imatinib (c)	[160–162]
AKT1	14q32.32	0	6	E17K	SqCC	MK2206 (2)	[100,163,164]
PIK3CA	3q26.3	1–3	3–7	E542K, E545K, H1047R	None	XL147 (2), BKM120 (2)	[87,100,165,166]
PIK3CA	3q26.3	6	33	Amplification	None	None	[87,165–167]
BRAF	7q34	2	1–3	G465V and L596R	None	Vemurafenib (2), Dasatinib (2), Dabrafenib (2)	[97,98,163,168,169]
PTEN	10q23.3	8–20	8–20	Deletion	None	None	[170,171]
PTEN	10q23.3	2	10	Mutation exon 9,20	None	None	[138,172]
MET	7q31	3–21	3–21	Amplification	None	Tivantinib (2), Onartuzumab (3), SU11274 (c)	[11,163,173–175]
MET	7q31	<5	<5	Mutation exon 14	None	Tivantinib (2), OSU11274 (c)	[87,163,173,176]
STK11	19p13	13–34	5–19	Exon 1 mutation	C, CS	None	[177,178]

AS: Asian, C: Caucasian, M: male, F: female, NS: never smoker, CS: current smoker, Y: young adult.
*: FDA approved, 2: Phase 2 trial, 3: Phase 3 trial, c: in vitro evidence, v: in vivo evidence.

5.1. AC predominant alterations

EGFR and *KRAS* mutations along with *EML4-ALK* fusions are the three most frequent driver alterations in AC, occurring with mutual exclusivity in approximately 35–40% of tumors (Fig. 1C and Table 2). Clinically, *EGFR* mutations are more prevalent in Asian female never smokers and are associated with a better prognosis while *KRAS* mutations are predictive of poor outcome, resistance to *EGFR* TKIs and are more common in smokers and Caucasians [90]. While there are currently no approved therapeutic agents for *KRAS* mutant tumors due to the difficulty of targeting KRAS itself, and debate surrounds whether KRAS should be included in molecular diagnostic panels [91] a number of combination therapies have recently shown efficacy in KRAS mutant tumors. In murine models of lung cancer, the combination of the MEK inhibitor (selumitinib) with either a BCL-XL (navitoclax) or PI3K (NVP-BKM120) inhibitor resulted in marked tumor regression, while in a randomized phase II study, the combination of selumetinib and docetaxel showed a clinical benefit in KRAS mutant tumors compared to placebo [92–94]. Despite the previous difficulties of targeting KRAS, these findings suggest that therapies targeting the multiple critical effectors of KRAS are effective and that targeted therapies for KRAS may soon be available. Other driver genes preferentially mutated in AC, but at a significantly lower frequency (1–4%) include *HER2* and *MAP2K1*/*MEK1* (Table 2) which are mutually exclusive of, *PIK3CA*, *BRAF*, *EGFR* and *KRAS* mutations [87].

5.2. SqCC predominant alterations

Fewer actionable alterations have been identified in SqCC and as a result targeted therapies for SqCC alterations have yet to be approved for clinical use. Recurrent alterations characteristic of SqCC include amplification of *SOX2*, *PIK3CA*, *PDGFRA* and *FGFR1* as well as mutation of *DDR2*, *AKT1* and *NRF2* (Fig. 1C) [95]. Despite a high frequency of *SOX2* and *PIK3CA* amplification (20–30% of cases), drugs targeting these alterations are not currently available. However, SOX2 inhibitors and inhibitors with activity against PIK3CA mutations such as NVP-BKM120, are currently under development. BMK120 is currently in phase II trials (NCT01297491) and is therefore one of the most advanced SqCC specific targeted therapies in development [96]. While inhibitors targeting, *PDGFRA* *FGFR1*, *DDR2* and *AKT1* are being development, clinical trials specifically enrolling lung SqCC patients with *FGFR1, PDGFRA* and *DDR2* mutations have not yet been reported. Recently, *IGF1R* and *EphA2* have emerged as potential therapeutic targets in SqCC [95], however drug specificity issues have hindered development of targeted agents against these alterations and as such they are not currently considered molecular subtypes.

5.3. Alterations common to both subtypes

Many alterations are observed at similar frequencies in both AC and SqCC (Table 2 and Fig. 1C), including *TP53*, *BRAF*, *PIK3CA*, *MET* and *STK11* mutations, loss of *PTEN* and amplification of *MET*, with *BRAF*, *PIK3CA*, and *MET* inhibitors already in development/trials. Although FDA approved targeted therapies against *BRAF* exist for the treatment of melanoma, only 10% of *BRAF* mutations in lung cancer are V600E, thus limiting the utility of most existing BRAF inhibitors [97,98]. Mutation of *TP53* is the most common mutation in both subtypes, occurring in more than 50% of samples, however, targeting *TP53* is inherently difficult due to the wide range of mutant proteins that exist and the multitude

of complex protein–protein interactions. Few effective targeted therapeutics against tumor suppressor genes exist, as they are significantly more difficult to target than a hyperactive oncogenes, although it is thought PTEN may be targetable in the near future [99,100].

5.4. Gene fusions

While gene fusions have been observed in both subtypes, they are more frequently found in AC (Fig. 1C). *EML4-ALK* translocations are the result of a small inversion within the short arm of chromosome 2 occurring in 3–7% of NSCLC [101–104]. To date more than 14 different *EML4-ALK* fusion variants have been identified [101], conferring resistance to *EGFR* TKIs, but sensitivity to ALK inhibitors such as crizotinib [105,106]. *ROS1* fusions are present in 1–2% of patients and have more than 10 different fusion partners (Table 2). Preliminary studies indicate that crizotinib has activity against ROS, however additional testing is still needed before crizotinib is approved for use in patients with ROS fusions. *RET* fusions, the newest class of gene fusion in lung cancer, are observed in 1–2% of patients, and typically involve fusion with *KIF5B* [54,88,107–113]. *RET-KIF5B* fusions are found predominantly in AC of never smokers and are mutually exclusive with mutations in *EGFR*, *KRAS* and *ALK* fusions [108,110,111]. Vandetanib, a multi-kinase inhibitor with anti-RET activity, has been approved by the FDA based on its efficiency in medullary thyroid carcinoma but its effectiveness in lung cancer is currently unknown [114]. Serine-threonine kinase and non-protein kinase fusions have also been identified in NSCLC, but only in single samples [23].

5.5. Clinical impact of targeted therapies

The success/benefits of targeted therapy have highlighted the importance of defining the molecular alterations within a tumor as well as histology. However, despite initial responses and improved outcomes associated with targeted therapies, the majority of patients develop resistance within a year or two, and all relapse, such that targeted agents for lung cancer are administered without curative intent. As such primary and acquired resistance remain major obstacles to the successful treatment of lung cancer. Mechanisms of resistance include, but are not limited to additional gene mutations, (ex: T790M in EGFR and L1196M and G1269A in ALK) gene amplification of the target and other genes (ex: MET), subtype conversion (NSCLC to SCLC) and activation of other signaling pathways, such as KIT, KRAS which act as a bypass mechanisms [115–117]. For EGFR TKIs, T790M mutations and MET amplification are the most common mechanisms of resistance, occurring in roughly 60% of cases, whereas for ALK, secondary mutations have been described in 30% of cases with resistance. A number of strategies to overcome resistance to targeted therapies have been developed. These include *MEK* [118] and heat shock protein inhibitors [119] to reverse acquired resistance to gefitinib and crizotinib respectively, dual kinase inhibitors such as lapatinib which targets both *EGFR* and *HER2* and have demonstrated effectiveness in breast tumors [120], and multidrug/multi-pathway targeting approaches [121]. Substantial effort has been directed toward overcoming resistance to therapy, and the specific details regarding mechanisms of resistance to TKIs, strategies to overcome resistance and development of second/third generation targeted therapies are reviewed in great detail elsewhere [117,121–124]. The application of repeat biopsies over the course of treatment is an ideal approach to studying mechanisms of resistance. However due to the practical limitations of repeat biopsies, this type of study is rare. The use of surrogate specimens such as tumor cells from malignant pleural effusions (MPE) (which occur in 15% of patients with advanced NSCLC) represents a possible alternative to repeat biopsies [125]. Pleural effusion fluid can be easily collected through relatively non-invasive procedures throughout the course of treatment and previous studies have shown high concordance between tumor and MPE tumor cell mutations [126]. Moreover, chemotherapy has been show to reach the pleural cavity, indicating tumor cells from MPE could be an extremely useful for studying mechanisms of resistance [127].

Notably, genomic profiling of SCLC has also revealed frequent alterations, e.g. P53, RB1 and EZH2, raising the potential of future development of targeted therapies blurring the separation of SCLC as a separate entity in the context of treatment design [128–130].

6. Emerging clinical practices

With the continued development of novel targeted therapeutics, genomic analyses of patient tumors to inform treatment selection will become routine clinical practice. However, due to the current costs of generating a complete tumor profile, most institutions only test for the most prominent alterations with indications for approved targeted therapies: *KRAS* and *EGFR* mutations and *EML4-ALK* fusions. Moving toward personalizing treatment, some of the major academic cancer centers have developed efficient, cost effective tumor genotyping protocols to screen patients for all actionable alterations, a few of which are discussed below.

The Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) trial completed in 2011, integrated real time molecular data in a clinical trial to identify specific patient populations likely to benefit from individualized treatment [131]. BATTLE established the feasibility of performing biopsies and real time biomarker analysis, and validated prespecified hypotheses regarding biomarkers and targeted agents while also identifying potential new predictive markers, thereby making substantial progress in the practice of personalized lung cancer treatment [131]. At Memorial Sloan Kettering, the Lung Cancer and Squamous Mutation Analysis Projects (LC-MAP and SQ-MAP) used multiplexed mass-spectrometry to test for alterations in targetable pathways, specifically *EGFR*, *KRAS*, *NRAS*, *BRAF*, *HER2*, *PIK3CA*, *MEK1*, *AKT1*, *PTEN*, *DDR2* mutations, *EML4-ALK* fusions and *FGFR1* amplification [132,133].

Building on the success of these initiatives and using the latest next-generation sequencing technology, MSKCC and MD Anderson have developed new cancer genomics pipelines; Integrated Mutation Profiling of Actionable Cancer Targets (IMPACT) which involves targeted exon sequencing of 275 cancer genes [134] and the Moon Shot Program which integrates early detection, smoking cessation, and genomic profiling with targeted drug discovery/repositioning (http://cancermoonshots.org/moon-shots/lung/). These comprehensive, high throughput approaches enable the detection of copy number alterations, genomic rearrangements and mutations with high coverage and sensitivity. Using these approaches, the therapeutic strategy with the greatest potential benefit can be administered to the patient, whether approved for clinical use or still in trial, bringing personalized treatment of lung cancer closer to reality.

Despite this progress, much work remains before genome characterization can be implemented into routine clinical decision making. Optimization of technologies, computational analysis and biological interpretation of sequencing results (passenger vs. driver mutations) in an efficient, cost effective manner with clinically useful turnaround times remain major challenges. With several different types of alterations to test for (deletions, insertions, mutations, amplifications and fusions) and more than a dozen actionable targets, a high throughput, highly sensitivity method is required. Moreover, technologies should be suitable for routine clinical specimens, some of which such as fine needle aspirates or biopsies can

have low tumor cell content. Although it is unlikely that cancer genome sequencing will ever serve as the exclusive test upon which clinical decisions are made, it may prove to be a powerful diagnostic and prognostic tool for guiding therapeutic decision making in the future.

7. Conclusion

The discovery and success of targeted therapies has launched a new era of lung cancer research focused on the detection and treatment of targetable alterations. Despite the continued identification of new driver alterations, half of NSCLC cases have no detected actionable alterations, and even for those targetable alterations, drug design and resistance remain major limitations to successful, curative treatment of lung cancer. While sequencing efforts continue to identify novel mutations in NSCLC, it is unlikely point mutations will characterize all tumors, emphasizing the need to look beyond protein coding genes, to ncRNAs and DNA methylation and how these different transcripts and alterations cooperate to deregulate pathways and signaling networks. Ongoing efforts toward further defining the landscape of genetic alterations in AC and SqCC and tumor heterogeneity will continue to improve our understanding of lung cancer biology, yielding novel therapeutic and diagnostic targets capable of improving the survival of NSCLC.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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References

[1] Society, A.C. Cancer Facts & Figures 2012; 2012.
[2] Statistics, C.C.S.s.S.C.o.C. Canadian Cancer Statistics 2012; 2012.
[3] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62(1):10–29.
[4] Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC, et al. Pathology and genetics of tumours of the lung, pleura, thymus and heart. World Health Organization classification of tumours; 2004.
[5] Mok TS, Thongrasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361(10):947–57.
[6] Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 2005;353(2):123–32.
[7] Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, et al. International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma. J Thorac Oncol 2011;6(2):244–85.
[8] Righi L, Graziano P, Fornari A, Rossi G, Barbareschi M, Cavazza A, et al. Immunohistochemical subtyping of nonsmall cell lung cancer not otherwise specified in fine-needle aspiration cytology: a retrospective study of 103 cases with surgical correlation. Cancer 2011;117(15):3416–23.
[9] Warth A, Muley T, Herpel E, Meister M, Herth FJ, Schirmacher P, et al. Large-scale comparative analyses of immunomarkers for diagnostic subtyping of non-small-cell lung cancer biopsies. Histopathology 2012;61(6):1017–25.
[10] Garraway LA, Sellers WR. Lineage dependency and lineage-survival oncogenes in human cancer. Nat Rev Cancer 2006;6(8):593–602.
[11] Weir BA, Woo MS, Getz G, Perner S, Ding L, Beroukhim R, et al. Characterizing the cancer genome in lung adenocarcinoma. Nature 2007;450(7171):893–8.
[12] Bass AJ, Watanabe H, Mermel CH, Yu SY, Perner S, Verhaak RG, et al. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat Genet 2009;41(11), 1238-U105.
[13] Bingle CD. Thyroid transcription factor-1. Int J Biochem Cell Biol 1997;29(12):1471–3.
[14] Ikeda K, Clark JC, Shaw-White JR, Stahlman MT, Boutell CJ, Whitsett JA. Gene structure and expression of human thyroid transcription factor-1 in respiratory epithelial cells. J Biol Chem 1995;270(14):8108–14.
[15] Que J, Luo X, Schwartz RJ, Hogan BL. Multiple roles for Sox2 in the developing and adult mouse trachea. Development 2009;136(11):1899–907.
[16] Wu M, Wang B, Gil J, Sabo E, Miller L, Gan L, et al. p63 and TTF-1 immunostaining. A useful marker panel for distinguishing small cell carcinoma of lung from poorly differentiated squamous cell carcinoma of lung. Am J Clin Pathol 2003;119(5):696–702.
[17] Kargi A, Gurel D, Tuna B. The diagnostic value of TTF-1 CK 5/6, and p63 immunostaining in classification of lung carcinomas. Appl Immunohistochem Mol Morphol 2007;15(4):415–20.
[18] Nonaka D. A study of DeltaNp63 expression in lung non-small cell carcinomas. Am J Surg Pathol 2012;36(6):895–9.
[19] Brcic L, Sherer CK, Shuai Y, Hornick JL, Chirieac LR, Dacic S. Morphologic and clinicopathologic features of lung squamous cell carcinomas expressing Sox2. Am J Clin Pathol 2012;138(5):712–8.
[20] Lockwood WW, Chari R, Coe BP, Thu KL, Garnis C, Malloff CA, et al. Integrative genomic analyses identify BRF2 as a novel lineage-specific oncogene in lung squamous cell carcinoma. PLoS Med 2010;7(7).
[21] Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers—a different disease. Nat Rev Cancer 2007;7(10):778–90.
[22] Govindan R, Ding L, Griffith M, Subramanian J, Dees ND, Kanchi KL, et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. Cell 2012;150(6):1121–34.
[23] Seo JS, Ju YS, Lee WC, Shin JY, Lee JK, Bleazard T, et al. The transcriptional landscape and mutational profile of lung adenocarcinoma. Genome Res 2012;22(11):2109–19.
[24] Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 2005;97(5):339–46.
[25] An SJ, Chen ZH, Su J, Zhang XC, Zhong WZ, Yang JJ, et al. Identification of enriched driver gene alterations in subgroups of non-small cell lung cancer patients based on histology and smoking status. PLoS One 2012;7(6):e40109.
[26] Thu KL, Vucic EA, Chari R, Zhang W, Lockwood WW, English JC, et al. Lung adenocarcinoma of never smokers and smokers harbor differential regions of genetic alteration and exhibit different levels of genomic instability. PLoS One 2012;7(3):e33003.
[27] Bhattacharjee A, Richards WG, Staunton J, Li C, Monti S, Vasa P, et al. Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. Proc Natl Acad Sci USA 2001;98(24):13790–5.
[28] Garber ME, Troyanskaya OG, Schluens K, Petersen S, Thaesler Z, Pacyna-Gengelbach M, et al. Diversity of gene expression in adenocarcinoma of the lung. Proc Natl Acad Sci USA 2001;98(24):13784–9.
[29] McDoniels-Silvers AL, Nimri CF, Stoner GD, Lubet RA, You M. Differential gene expression in human lung adenocarcinomas and squamous cell carcinomas. Clin Cancer Res 2002;8(4):1127–38.
[30] McDoniels-Silvers AL, Stoner GD, Lubet RA, You M. Differential expression of critical cellular genes in human lung adenocarcinomas and squamous cell carcinomas in comparison to normal lung tissues. Neoplasia 2002;4(2):141–50.
[31] Nacht M, Dracheva T, Gao Y, Fujii T, Chen Y, Player A, et al. Molecular characteristics of non-small cell lung cancer. Proc Natl Acad Sci USA 2001;98(26):15203–8.
[32] Wilkerson MD, Yin X, Hoadley KA, Liu Y, Hayward MC, Cabanski CR, et al. Lung squamous cell carcinoma mRNA expression subtypes are reproducible, clinically important, and correspond to normal cell types. Clin Cancer Res 2010;16(19):4864–75.
[33] Hou J, Aerts J, den Hamer B, van Ijcken W, den Bakker M, Riegman P, et al. Gene expression-based classification of non-small cell lung carcinomas and survival prediction. PLoS One 2010;5(4):e10312.
[34] Yamagata N, Shyr Y, Yanagisawa K, Edgerton M, Dang TP, Gonzalez A, et al. A training-testing approach to the molecular classification of resected non-small cell lung cancer. Clin Cancer Res 2003;9(13):4695–704.
[35] Virtanen C, Ishikawa Y, Honjoh D, Kimura M, Shimane M, Miyoshi T, et al. Integrated classification of lung tumors and cell lines by expression profiling. Proc Natl Acad Sci USA 2002;99(19):12357–62.
[36] Rohrbeck A, Neukirchen J, Rosskopf M, Pardillos GG, Geddert H, Schwalen A, et al. Gene expression profiling for molecular distinction and characterization of laser captured primary lung cancers. J Transl Med 2008;6:69.
[37] Beer DG, Kardia SL, Huang CC, Giordano TJ, Levin AM, Misek DE, et al. Gene-expression profiles predict survival of patients with lung adenocarcinoma. Nat Med 2002;8(8):816–24.
[38] Giordano TJ, Shedden KA, Schwartz DR, Kuick R, Taylor JMG, Lee N, et al. Organ-specific molecular classification of primary lung, colon, and ovarian adenocarcinomas using gene expression profiles. Am J Pathol 2001;159(4):1231–8.
[39] Balsara BR, Testa JR. Chromosomal imbalances in human lung cancer. Oncogene 2002;21(45):6877–83.
[40] Pei J, Balsara BR, Li W, Litwin S, Gabrielson E, Feder M, et al. Genomic imbalances in human lung adenocarcinomas and squamous cell carcinomas. Genes Chromosomes Cancer 2001;31(3):282–7.
[41] Sy SM, Wong N, Lee TW, Tse G, Mok TS, Fan B, et al. Distinct patterns of genetic alterations in adenocarcinoma and squamous cell carcinoma of the lung. Eur J Cancer 2004;40(7):1082–94.

[42] Tonon G, Wong KK, Maulik G, Brennan C, Feng B, Zhang Y, et al. High-resolution genomic profiles of human lung cancer. Proc Natl Acad Sci USA 2005;102(27):9625–30.
[43] Yakut T, Schulten HJ, Demir A, Frank D, Danner B, Egeli U, et al. Assessment of molecular events in squamous and non-squamous cell lung carcinoma. Lung Cancer 2006;54(3):293–301.
[44] Bjorkqvist AM, Husgafvel-Pursiainen K, Anttila S, Karjalainen A, Tammilehto L, Mattson K, et al. DNA gains in 3q occur frequently in squamous cell carcinoma of the lung, but not in adenocarcinoma. Genes Chromosomes Cancer 1998;22(1):79–82.
[45] Garnis C, Lockwood WW, Vucic E, Ge Y, Girard L, Minna JD, et al. High resolution analysis of non-small cell lung cancer cell lines by whole genome tiling path array CGH. Int J Cancer 2006;118(6):1556–64.
[46] Luk C, Tsao MS, Bayani J, Shepherd F, Squire JA. Molecular cytogenetic analysis of non-small cell lung carcinoma by spectral karyotyping and comparative genomic hybridization. Cancer Genet Cytogenet 2001;125(2):87–99.
[47] Massion PP, Kuo WL, Stokoe D, Olshen AB, Treseler PA, Chin K, et al. Genomic copy number analysis of non-small cell lung cancer using array comparative genomic hybridization: implications of the phosphatidylinositol 3-kinase pathway. Cancer Res 2002;62(13):3636–40.
[48] Petersen I, Bujard M, Petersen S, Wolf G, Goeze A, Schwendel A, et al. Patterns of chromosomal imbalances in adenocarcinoma and squamous cell carcinoma of the lung. Cancer Res 1997;57(12):2331–5.
[49] Staaf J, Isaksson S, Karlsson A, Jonsson M, Johansson L, Jonsson P, et al. Landscape of somatic allelic imbalances and copy number alterations in human lung carcinoma. Int J Cancer 2012;132(9):2020–31.
[50] Hammerman PS, Lawrence MS, Voet D, Jing R, Cibulskis K, Sivachenko A, et al. Comprehensive genomic characterization of squamous cell lung cancers. Nature 2012;489(7417):519–25.
[51] Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature 2008;455(7216):1069–75.
[52] Imielinski M, Berger AH, Hammerman PS, Hernandez B, Pugh TJ, Hodis E, et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell 2012;150(6):1107–20.
[53] Liu J, Lee W, Jiang Z, Chen Z, Jhunjhunwala S, Haverty PM, et al. Genome and transcriptome sequencing of lung cancers reveal diverse mutational and splicing events. Genome Res 2012;22(12):2315–27.
[54] Ju YS, Lee WC, Shin JY, Lee S, Bleazard T, Won JK, et al. A transforming KIF5B and RET gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing. Genome Res 2012;22(3):436–45.
[55] Lee W, Jiang Z, Liu J, Haverty PM, Guan Y, Stinson J, et al. The mutation spectrum revealed by paired genome sequences from a lung cancer patient. Nature 2010;465(7297):7–473.
[56] Liu P, Morrison C, Wang L, Xiong D, Vedell P, Cui P, et al. Identification of somatic mutations in non-small cell lung carcinomas using whole-exome sequencing. Carcinogenesis 2012;33(7):1270–6.
[57] Kan Z, Jaiswal BS, Stinson J, Janakiraman V, Bhatt D, Stern HM, et al. Diverse somatic mutation patterns and pathway alterations in human cancers. Nature 2010;466(7308):73–869.
[58] Baylin SB. The cancer epigenome: its origins, contributions to tumorigenesis, and translational implications. Proc Am Thorac Soc 2012;9(2):64–5.
[59] Tessema M, Belinsky SA. Mining the epigenome for methylated genes in lung cancer. Proc Am Thorac Soc 2008;5(8):806–10.
[60] Heller G, Zielinski CC, Zochbauer-Muller S. Lung cancer: from single-gene methylation to methylome profiling. Cancer Metastasis Rev 2010;29(1):95–107.
[61] Shiraishi M, Sekiguchi A, Oates AJ, Terry MJ, Miyamoto Y. HOX gene clusters are hotspots of de novo methylation in CpG islands of human lung adenocarcinomas. Oncogene 2002;21(22):3659–62.
[62] Zochbauer-Muller S, Fong KM, Virmani AK, Geradts J, Gazdar AF, Minna JD. Aberrant promoter methylation of multiple genes in non-small cell lung cancers. Cancer Res 2001;61(1):249–55.
[63] Toyooka S, Tokumo M, Shigematsu H, Matsuo K, Asano H, Tomii K, et al. Mutational and epigenetic evidence for independent pathways for lung adenocarcinomas arising in smokers and never smokers. Cancer Res 2006;66(3):5–1371.
[64] Heller G, Babinsky VN, Ziegler B, Weinzierl M, Noll C, Altenberger C, et al. Genome-wide CpG island methylation analyses in non-small cell lung cancer patients. Carcinogenesis 2012;34(3):513–21.
[65] Lockwood WW, Wilson IM, Coe BP, Chari R, Pikor LA, Thu KL, et al. Divergent genomic and epigenomic landscapes of lung cancer subtypes underscore the selection of different oncogenic pathways during tumor development. PLoS One 2012;7(5):e37775.
[66] Selamat SA, Chung BS, Girard L, Zhang W, Zhang Y, Campan M, et al. Genome-scale analysis of DNA methylation in lung adenocarcinoma and integration with mRNA expression. Genome Res 2012;22(7):211–1197.
[67] Birney E, Stamatoyannopoulos JA, Dutta A, Guigo R, Gingeras TR, Margulies EH, et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 2007;447(7146):799–816.
[68] Ponting CP, Belgard TG. Transcribed dark matter: meaning or myth? Hum Mol Genet 2010;19(R2):R162–8.
[69] Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, et al. The transcriptional landscape of the mammalian genome. Science 2005;309(5740):1559–63.
[70] Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006;6(11):857–66.
[71] Lebanony D, Benjamin H, Gilad S, Ezagouri M, Dov A, Ashkenazi K, et al. Diagnostic assay based on hsa-miR-205 expression distinguishes squamous from nonsquamous non-small-cell lung carcinoma. J Clin Oncol 2009;27(12):7–2030.
[72] Dacic S, Kelly L, Shuai Y, Nikiforova MN. miRNA expression profiling of lung adenocarcinomas: correlation with mutational status. Mod Pathol 2010;23(12):82–1577.
[73] Seike M, Goto A, Okano T, Bowman ED, Schetter AJ, Horikawa I, et al. MiR-21 is an EGFR-regulated anti-apoptotic factor in lung cancer in never-smokers. Proc Natl Acad Sci USA 2009;106(29):90–12085.
[74] Tinzl M, Marberger M, Horvath S, Chypre C. DD3PCA3 RNA analysis in urine—a new perspective for detecting prostate cancer. Eur Urol 2004;46(2):6–182, discussion 187.
[75] Panzitt K, Tschernatsch MM, Guelly C, Moustafa T, Stradner M, Strohmaier HM, et al. Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA. Gastroenterology 2007;132(1):42–330.
[76] Ji P, Diederichs S, Wang W, Boing S, Metzger R, Schneider PM, et al. MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene 2003;22(39):41–8031.
[77] Chari R, Thu KL, Wilson IM, Lockwood WW, Lonergan KM, Coe BP, et al. Integrating the multiple dimensions of genomic and epigenomic landscapes of cancer. Cancer Metastasis Rev 2010;29(1):73–93.
[78] Daraselia N, Wang Y, Budoff A, Lituev A, Potapova O, Vansant G, et al. Molecular signature and pathway analysis of human primary squamous and adenocarcinoma lung cancers. Am J Cancer Res 2012;2(1):93–103.
[79] Gettinger S, Lynch T. A decade of advances in treatment for advanced non-small cell lung cancer. Clin Chest Med 2011;32(4):51–839.
[80] Johnson DH, Fehrenbacher L, Novotny WF, Herbst RS, Nemunaitis JJ, Jablons DM, et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. J Clin Oncol 2004;22(11):91–2184.
[81] Scagliotti G, Hanna N, Fossella F, Sugarman K, Blatter J, Peterson P, et al. The differential efficacy of pemetrexed according to NSCLC histology: a review of two Phase III studies. Oncologist 2009;14(3):63–253.
[82] Hanauske AR, Eismann U, Oberschmidt O, Pospisil H, Hoffmann S, Hanauske-Abel H, et al. In vitro chemosensitivity of freshly explanted tumor cells to pemetrexed is correlated with target gene expression. Invest New Drugs 2007;25(5):23–417.
[83] Igawa S, Ryuge S, Wada M, Otani S, Maki S, Takakura A, et al. Pemetrexed for previously treated patients with non-small cell lung cancer and differences in efficacy according to thymidylate synthase expression. Chemotherapy 2012;58(4):20–313.
[84] Ceppi P, Volante M, Saviozzi S, Rapa I, Novello S, Cambieri A, et al. Squamous cell carcinoma of the lung compared with other histotypes shows higher messenger RNA and protein levels for thymidylate synthase. Cancer 2006;107(7):96–1589.
[85] Heist RS, Engelman JA. SnapShot: non-small cell lung cancer. Cancer Cell 2012;21(3):448 e2.
[86] Lovly CM, Carbone DP. Lung cancer in 2010: one size does not fit all. Nat Rev Clin Oncol 2011;8(2):68–70.
[87] Pao W, Girard N. New driver mutations in non-small-cell lung cancer. Lancet Oncol 2011;12(2):80–175.
[88] Pao W, Hutchinson KE. Chipping away at the lung cancer genome. Nat Med 2012;18(3):349–51.
[89] Toyooka S, Mitsudomi T, Soh J, Aokage K, Yamane M, Oto T, et al. Molecular oncology of lung cancer. Gen Thorac Cardiovasc Surg 2011;59(8):37–527.
[90] Shigematsu H, Gazdar AF. Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. Int J Cancer 2006;118(2):62–257.
[91] Roberts PJ, Stinchcombe TE. KRAS mutation: should we test for it, and does it matter? J Clin Oncol 2013;31(8):21–1112.
[92] Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, et al. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. Nat Med 2008;14(12):6–1351.
[93] Janne PA, Shaw AT, Pereira JR, Jeannin G, Vansteenkiste J, Barrios C, et al. Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. Lancet Oncol 2013;14(1):38–47.
[94] Corcoran RB, Cheng KA, Hata AN, Faber AC, Ebi H, Coffee EM, et al. Synthetic lethal interaction of combined BCL-XL and MEK inhibition promotes tumor regressions in KRAS mutant cancer models. Cancer Cell 2013;23(1):8–121.
[95] Heist RS, Sequist LV, Engelman JA. Genetic changes in squamous cell lung cancer: a review. J Thorac Oncol 2012;7(5):33–924.
[96] Narasimhan K, Pillay S, Bin Ahmad NR, Bikadi Z, Hazai E, Yan L, et al. Identification of a polyoxometalate inhibitor of the DNA binding activity of Sox2. ACS Chem Biol 2011;6(6):81–573.
[97] Brose MS, Volpe P, Feldman M, Kumar M, Rishi I, Gerrero R, et al. BRAF and RAS mutations in human lung cancer and melanoma. Cancer Res 2002;62(23):6997–7000.
[98] Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417(6892):949–54.

[99] Shackelford DB, Shaw RJ. The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. Nat Rev Cancer 2009;9(8):563–75.
[100] Drilon A, Rekhtman N, Ladanyi M, Paik P. Squamous-cell carcinomas of the lung: emerging biology, controversies, and the promise of targeted therapy. Lancet Oncol 2012;13(10):e418–26.
[101] Horn L, Pao W. EML4-ALK: honing in on a new target in non-small-cell lung cancer. J Clin Oncol 2009;27(26):4232–5.
[102] Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, Holmes AJ. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin Cancer Res 2008;14(13):4275–83.
[103] Choi YL, Takeuchi K, Soda M, Inamura K, Togashi Y, Hatano S, et al. Identification of novel isoforms of the EML4-ALK transforming gene in non-small cell lung cancer. Cancer Res 2008;68(13):4971–6.
[104] Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448(7153):561–6.
[105] Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol 2009;27(26):4247–53.
[106] Wang R, Pan Y, Li C, Hu H, Zhang Y, Li H, et al. The use of quantitative real-time reverse transcriptase PCR for 5′ and 3′ portions of ALK transcripts to detect ALK rearrangements in lung cancers. Clin Cancer Res 2012;18(17):4725–32.
[107] Wang R, Hu H, Pan Y, Li Y, Ye T, Li C, et al. RET fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer. J Clin Oncol 2012;30(35):4352–9.
[108] Kohno T, Ichikawa H, Totoki Y, Yasuda K, Hiramoto M, Nammo T, et al. KIF5B-RET fusions in lung adenocarcinoma. Nat Med 2012;18(3):375–7.
[109] Li F, Feng Y, Fang R, Fang Z, Xia J, Han X, et al. Identification of RET gene fusion by exon array analyses in "pan-negative" lung cancer from never smokers. Cell Res 2012;22(5):928–31.
[110] Lipson D, Capelletti M, Yelensky R, Otto G, Parker A, Jarosz M, et al. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. Nat Med 2012;18(3):382–4.
[111] Takeuchi K, Soda M, Togashi Y, Suzuki R, Sakata S, Hatano S, et al. RET ROS1 and ALK fusions in lung cancer. Nat Med 2012;18(3):378–81.
[112] Chao BH, Briesewitz R, Villalona-Calero MA. RET fusion genes in non-small-cell lung cancer. J Clin Oncol 2012;30(35):4439–41.
[113] Sablin EP. Kinesins and microtubules: their structures and motor mechanisms. Curr Opin Cell Biol 2000;12(1):35–41.
[114] Wells Jr SA, Gosnell JE, Gagel RF, Moley J, Pfister D, Sosa JA, et al. Vandetanib for the treatment of patients with locally advanced or metastatic hereditary medullary thyroid cancer. J Clin Oncol 2010;28(5):767–72.
[115] Doebele RC, Pilling AB, Aisner DL, Kutateladze TG, Le AT, Weickhardt AJ, et al. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clin Cancer Res 2012;18(5):1472–82.
[116] Katayama R, Shaw AT, Khan TM, Mino-Kenudson M, Solomon BJ, Halmos B, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung cancers. Sci Transl Med 2012;4(120):120ra17.
[117] Tartarone A, Lazzari C, Lerose R, Conteduca V, Improta G, Zupa A, et al. Mechanisms of resistance to EGFR tyrosine kinase inhibitors gefitinib/erlotinib and to ALK inhibitor crizotinib. Lung Cancer 2013;81(3):328–36.
[118] Sequist LV, Gettinger S, Senzer NN, Martins RG, Janne PA, Lilenbaum R, et al. Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non-small-cell lung cancer. J Clin Oncol 2010;28(33):4953–60.
[119] Huang MH, Lee JH, Chang YJ, Tsai HH, Lin YL, Lin AM, et al. MEK inhibitors reverse resistance in epidermal growth factor receptor mutation lung cancer cells with acquired resistance to gefitinib. Mol Oncol 2012;7(1):112–20.
[120] Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 2006;355(26):2733–43.
[121] Pao W, Chmielecki J. Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. Nat Rev Cancer 2010;10(11):760–74.
[122] Lin L, Bivona TG. Mechanisms of resistance to epidermal growth factor receptor inhibitors and novel therapeutic strategies to overcome resistance in NSCLC patients. Chemother Res Pract 2012;2012:817297.
[123] Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3(75).
[124] Oxnard GR. Strategies for overcoming acquired resistance to epidermal growth factor receptor: targeted therapies in lung cancer. Arch Pathol Lab Med 2012;136(10):1205–9.
[125] Basak SK, Veena MS, Oh S, Huang G, Srivatsan E, Huang M, et al. The malignant pleural effusion as a model to investigate intratumoral heterogeneity in lung cancer. PLoS One 2009;4(6):e5884.
[126] Yeo CD, Kim JW, Kim KH, Ha JH, Rhee CK, Kim SJ, et al. Detection and comparison of EGFR mutations in matched tumor tissues, cell blocks, pleural effusions, and sera from patients with NSCLC with malignant pleural effusion, by PNA clamping and direct sequencing. Lung Cancer 2013;81(2):207–12.
[127] Masago K, Togashi Y, Fukudo M, Terada T, Irisa K, Sakamori Y, et al. Plasma and pleural fluid pharmacokinetics of erlotinib and its active metabolite OSI-420 in patients with non-small-cell lung cancer with pleural effusion. Clin Lung Cancer 2011;12(5):307–12.
[128] Byers LA, Wang J, Nilsson MB, Fujimoto J, Saintigny P, Yordy J, et al. Proteomic profiling identifies dysregulated pathways in small cell lung cancer and novel therapeutic targets including PARP1. Cancer Discov 2012;2(9):798–811.
[129] Peifer M, Fernandez-Cuesta L, Sos ML, George J, Seidel D, Kasper LH, et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. Nat Genet 2012;44(10):1104–10.
[130] Wistuba II, Gazdar AF, Minna JD. Molecular genetics of small cell lung carcinoma. Semin Oncol 2001;28(2 Suppl. 4):3–13.
[131] Kim ES, Herbst RS, Wistuba II, Lee JJ, Blumenschein Jr GR, Tsao A, et al. The BATTLE trial: personalizing therapy for lung cancer. Cancer Discov 2011;1(1):44–53.
[132] Kris MG, Lau CY, Ang D, Brzostowski E, Riely GJ, Rusch VW, et al. Initial results of LC-MAP: an institutional program to routinely profile tumor specimens for the presence of mutations in targetable pathways in all patients with lung adenocarcinoma. In: 2010 ASCO Annual Meeting. 2010.
[133] Paik PK, Hasanovic A, Wang L, Rekhtman N, Ladanyi M, Kris MG. Multiplex testing for driver mutations in squamous cell carcinomas of the lung. In: 2012 ASCO Annual Meeting. 2012.
[134] Wagle N, Berger MF, Davis MJ, Blumenstiel B, Defelice M, Pochanard P, et al. High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. Cancer Discov 2012;2(1):82–93.
[135] Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11(2):121–8.
[136] Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304(5676):1497–500.
[137] Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350(21):2129–39.
[138] Forbes SA, Bindal N, Bamford S, Cole C, Kok CY, Beare D, et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res 2011;39(Database issue):D945–50.
[139] Emery CM, Vijayendran KG, Zipser MC, Sawyer AM, Niu L, Kim JJ, et al. MEK1 mutations confer resistance to MEK and B-RAF inhibition. Proc Natl Acad Sci USA 2009;106(48):20411–6.
[140] Marks JL, Gong Y, Chitale D, Golas B, McLellan MD, Kasai Y, et al. Novel MEK1 mutation identified by mutational analysis of epidermal growth factor receptor signaling pathway genes in lung adenocarcinoma. Cancer Res 2008;68(14):5524–8.
[141] Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363(18):1693–703.
[142] Takeuchi K, Choi YL, Soda M, Inamura K, Togashi Y, Hatano S, et al. Multiplex reverse transcription-PCR screening for EML4-ALK fusion transcripts. Clin Cancer Res 2008;14(20):6618–24.
[143] Takeuchi K, Choi YL, Togashi Y, Soda M, Hatano S, Inamura K, et al. KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. Clin Cancer Res 2009;15(9):3143–9.
[144] Bergethon K, Shaw AT, Ou SH, Katayama R, Lovly CM, McDonald NT, et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol 2012;30(8):863–70.
[145] Janne PA, Meyerson M. ROS1 rearrangements in lung cancer: a new genomic subset of lung adenocarcinoma. J Clin Oncol 2012;30(8):878–9.
[146] Rikova K, Guo A, Zeng Q, Possemato A, Yu J, Haack H, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 2007;131(6):1190–203.
[147] Suehara Y, Arcila ME, Wang L, Hasanovic A, Ang DC, Ito T, et al. Identification of KIF5B-RET and GOPC-ROS1 fusions in lung adenocarcinomas through a comprehensive mRNA-based screen for tyrosine kinase fusions. Clin Cancer Res 2012;18(24):6599–608.
[148] Rimkunas VM, Crosby KE, Li D, Hu Y, Kelly ME, Gu TL, et al. Analysis of receptor tyrosine kinase ROS1-positive tumors in non-small cell lung cancer: identification of a FIG-ROS1 fusion. Clin Cancer Res 2012;18(16):4449–57.
[149] Clinical Trials Registry and Database; 2013 [cited 2013; Available from: http://clinicaltrials.gov].
[150] Matsubara D, Kanai Y, Ishikawa S, Ohara S, Yoshimoto T, Sakatani T, et al. Identification of CCDC6-RET fusion in the human lung adenocarcinoma cell line, LC-2/ad. J Thorac Oncol 2012;7(12):1872–6.
[151] Ramos AH, Dutt A, Mermel C, Perner S, Cho J, Lafargue CJ, et al. Amplification of chromosomal segment 4q12 in non-small cell lung cancer. Cancer Biol Ther 2009;8(21):2042–50.
[152] Kim HS, Mitsudomi T, Soo RA, Cho BC. Personalized therapy on the horizon for squamous cell carcinoma of the lung. Lung Cancer 2013;80(3):249–55.
[153] Weiss J, Sos ML, Seidel D, Peifer M, Zander T, Heuckmann JM, et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med 2010;2(62).
[154] Heist RS, Mino-Kenudson M, Sequist LV, Tammireddy S, Morrissey L, Christiani DC. FGFR1 amplification in squamous cell carcinoma of the lung. J Thorac Oncol 2012;7(12):1775–80.
[155] Dutt A, Ramos AH, Hammerman PS, Mermel C, Cho J, Sharifnia T, et al. Inhibitor-sensitive FGFR1 amplification in human non-small cell lung cancer. PLoS One 2011;6(6):e20351.

[156] Shibata T, Ohta T, Tong KI, Kokubu A, Odogawa R, Tsuta K, et al. Cancer related mutations in NRF2 impair its recognition by Keap1-Cul3 E3 ligase and promote malignancy. Proc Natl Acad Sci USA 2008;105(36):13568–73.
[157] Kim YR, Oh JE, Kim MS, Kang MR, Park SW, Han JY, et al. Oncogenic NRF2 mutations in squamous cell carcinomas of oesophagus and skin. J Pathol 2010;220(4):446–51.
[158] Singh A, Misra V, Thimmulappa RK, Lee H, Ames S, Hoque MO, et al. Dysfunctional KEAP1-NRF2 interaction in non-small-cell lung cancer. PLoS Med 2006;3(10):e420.
[159] Singh A, Bodas M, Wakabayashi N, Bunz F, Biswal S. Gain of Nrf2 function in non-small-cell lung cancer cells confers radioresistance. Antioxid Redox Signal 2010;13(11):1627–37.
[160] Johnson FM, Bekele BN, Feng L, Wistuba I, Tang XM, Tran HT, et al. Phase II study of dasatinib in patients with advanced non-small-cell lung cancer. J Clin Oncol 2010;28(30):4609–15.
[161] Day E, Waters B, Spiegel K, Alnadaf T, Manley PW, Buchdunger E, et al. Inhibition of collagen-induced discoidin domain receptor 1 and 2 activation by imatinib, nilotinib and dasatinib. Eur J Pharmacol 2008;599(1–3):44–53.
[162] Hammerman PS, Sos ML, Ramos AH, Xu C, Dutt A, Zhou W, et al. Mutations in the DDR2 kinase gene identify a novel therapeutic target in squamous cell lung cancer. Cancer Discov 2011;1(1):78–89.
[163] Perez-Moreno P, Brambilla E, Thomas R, Soria JC. Squamous cell carcinoma of the lung: molecular subtypes and therapeutic opportunities. Clin Cancer Res 2012;18(9):2443–51.
[164] Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, et al. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature 2007;448(7152):439–44.
[165] Ikenoue T, Kanai F, Hikiba Y, Obata T, Tanaka Y, Imamura J, et al. Functional analysis of PIK3CA gene mutations in human colorectal cancer. Cancer Res 2005;65(11):4562–7.
[166] Kang S, Bader AG, Vogt PK. Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. Proc Natl Acad Sci USA 2005;102(3):802–7.
[167] Yamamoto H, Shigematsu H, Nomura M, Lockwood WW, Sato M, Okumura N, et al. PIK3CA mutations and copy number gains in human lung cancers. Cancer Res 2008;68(17):6913–21.
[168] Naoki K, Chen TH, Richards WG, Sugarbaker DJ, Meyerson M. Missense mutations of the BRAF gene in human lung adenocarcinoma. Cancer Res 2002;62(23):7001–3.
[169] Leicht DT, Balan V, Kaplun A, Singh-Gupta V, Kaplun L, Dobson M, et al. Raf kinases: function, regulation and role in human cancer. Biochim Biophys Acta 2007;1773(8):1196–212.
[170] Kohno T, Takahashi M, Manda R, Yokota J. Inactivation of the PTEN/MMAC1/TEP1 gene in human lung cancers. Genes Chromosomes Cancer 1998;22(2):152–6.
[171] Marsit CJ, Zheng S, Aldape K, Hinds PW, Nelson HH, Wiencke JK, et al. PTEN expression in non-small-cell lung cancer: evaluating its relation to tumor characteristics, allelic loss, and epigenetic alteration. Hum Pathol 2005;36(7):768–76.
[172] Jin G, Kim MJ, Jeon HS, Choi JE, Kim DS, Lee EB, et al. PTEN mutations and relationship to EGFR, ERBB2 KRAS, and TP53 mutations in non-small cell lung cancers. Lung Cancer 2010;69(3):279–83.
[173] Ma PC, Jagadeeswaran R, Jagadeesh S, Tretiakova MS, Nallasura V, Fox EA, et al. Functional expression and mutations of c-Met and its therapeutic inhibition with SU11274 and small interfering RNA in non-small cell lung cancer. Cancer Res 2005;65(4):1479–88.
[174] Onozato R, Kosaka T, Kuwano H, Sekido Y, Yatabe Y, Mitsudomi T, et al. Activation of MET by gene amplification or by splice mutations deleting the juxtamembrane domain in primary resected lung cancers. J Thorac Oncol 2009;4(1):5–11.
[175] Lutterbach B, Zeng Q, Davis LJ, Hatch H, Hang G, Kohl NE, et al. Lung cancer cell lines harboring MET gene amplification are dependent on Met for growth and survival. Cancer Res 2007;67(5):2081–8.
[176] Kong-Beltran M, Seshagiri S, Zha J, Zhu W, Bhawe K, Mendoza N, et al. Somatic mutations lead to an oncogenic deletion of met in lung cancer. Cancer Res 2006;66(1):283–9.
[177] Koivunen JP, Kim J, Lee J, Rogers AM, Park JO, Zhao X, et al. Mutations in the LKB1 tumour suppressor are frequently detected in tumours from Caucasian but not Asian lung cancer patients. Br J Cancer 2008;99(2):245–52.
[178] Ji H, Ramsey MR, Hayes DN, Fan C, McNamara K, Kozlowski P. LKB1 modulates lung cancer differentiation and metastasis. Nature 2007;448(7155):807–10.