

MINI-REVIEW

Clinical Efficacy and Possible Applications of Genomics in Lung Cancer

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Abstract

The heterogeneous nature of lung cancer has become increasingly apparent since introduction of molecular classification. In general, advanced lung cancer is an aggressive malignancy with a poor prognosis. Activating alterations in several potential driver oncogenic genes have been identified, including EGFR, ROS1 and ALK and understanding of their molecular mechanisms underlying development, progression, and survival of lung cancer has led to the design of personalized treatments that have produced superior clinical outcomes in tumours harbouring these mutations. In light of the tsunami of new biomarkers and targeted agents, next generation sequencing testing strategies will be more appropriate in identifying the patients for each therapy and enabling personalized patients care. The challenge now is how best to interpret the results of these genomic tests, in the context of other clinical data, to optimize treatment choices. In genomic era of cancer treatment, the traditional one-size-fits-all paradigm is being replaced with more effective, personalized oncologic care. This review provides an overview of lung cancer genomics and personalized treatment.

Keywords: Lung cancer - genomics - personalized treatment

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Introduction

Regardless of histologic subtype, lung cancer is one of the most genomically diverse and unstable of all cancers, creating terrific challenges for both prevention and treatment strategies (Larsen and Minna, 2011; DeSantis et al., 2012). Nevertheless, this same biologic diversity provides a number of opportunities for utilization of interpatient tumor heterogeneity by ungrouping lung cancer into a variety of molecularly defined subsets for which mutations and/or abnormal gene expressions drive disease progression, survival and can serve as druggable targets (Sun et al., 2007; Ding et al., 2008; Pao and Girard, 2011).

Although the transition from traditional to mechanism-based, molecular marker driven therapeutic decision making is in its early phases, new drug classes have already changed the paradigm for the management of advanced-stage lung cancer (Gandara et al., 2009; 2012). A well studied example is the gain-of-function tyrosine kinase activating epidermal growth factor receptor (EGFR) mutations as the best predictive biomarker over clinicopathologic features in predicting tumor response and progression free survival to EGFR tyrosine kinase inhibitors (TKIs) (Mok et al., 2009; Mitsudomi et al., 2010; Maemondo., 2010; Zhou et al., 2011; Rosell et al., 2012). Similarly, gain-of-function tyrosine kinase activating ALK gene rearrangements, strong predictive biomarker in predicting tumor response and progression free survival

to the first-in-class ALK TKI crizotinib (Kwak et al., 2010). US FDA granted approval of the first-in-class ALK inhibitor crizotinib for treatment of ALK-positive advanced NSCLCs (US Food and Drug Administration). Subsequently, both the National Comprehensive Cancer Network (NCCN) and American Society of Clinical Oncology guidelines recommended EGFR mutation and ALK gene rearrangements testing on all NSCLCs that contain an adenocarcinoma component, regardless of histologic grade or dominant histologic subtype (Keedy et al., 2011; National Comprehensive Cancer Network, 2015). EGFR mutation and ALK testing is not recommended for pure squamous cell carcinomas, pure small-cell carcinomas, or pure neuroendocrine carcinomas (Field et al., 2011).

Although clinical utility of single gene biomarker has already proven successful in guiding selection of targeted therapies in non small cell lung cancer (NSCLC) (Von et al., 2010; Paik et al., 2012). Recent genomic studies have revealed that distinct genomic abnormalities are present in adenocarcinomas and squamous cell carcinomas (Jemal et al., 2011; Govindan et al., 2012), providing opportunities for developing novel molecularly targeted and biomarker driven therapeutic strategies for specific molecular subsets of patients.

Several high throughput studies identified new susceptibility loci for lung cancer, which highlights the genetic heterogeneity of lung cancer susceptibility among different ethnic populations (Brian et al., 2012). However,

most of studies of genome-wide alterations in lung cancer have been carried out in patients from Western countries, and to our best knowledge none of such studies have been done in Asian population. Moreover, individual tumors vary widely in evolution and behavior (i.e., tumor dormancy, local growth, distant dissemination, treatment response, and relapse). Thus, individuals who share the same histopathological stages, grades, and receive the same treatments could have tumors with completely different evolutionary histories and clinical outcomes. Moreover, some patients with non-aggressive disease can live over 5 years, whereas others die from metastatic disease within 2-3 years after diagnosis. Several lines of evidence have suggested that the heterogeneous clinical behavior of lung cancer is the result of different underlying molecular mechanisms during tumor progression (Jemal et al., 2010).

Shift from Microarrays to Next Generation Sequencing

Microarray technology has been invaluable tools for studying of complex biological mechanisms, resulting in a new understanding of lung cancer pathogenesis and providing a foundation for the generation of novel biomarkers for diagnosis, prognosis and the prediction of treatment responses. Although microarray based studies have contributed significantly to our understanding of the development and progression of human cancer, these technologies have several major limitations, including their inability to detect structural genomic aberration, alternative splice mechanism etc. The rapid development of next-generation sequencing (NGS) technologies has overcome many of these problems (Morozova and Marra 2008; Voelkerding et al., 2009; Wang et al., 2009). NGS permits the investigation of an entire cancer genome and transcriptome with unprecedented resolution and throughput (Bell, 2010; Ding et al., 2010; Oszlak and Milos, 2011). The first whole cancer genome sequence was reported in 2008, which compared the DNA sequence from an acute myeloid leukemia with that from normal skin from the same patient (Meyerson et al., 2010). Subsequently, whole-genome sequencing has been used to identify a wide range of genomic alterations, such as nucleotide substitutions, copy number alterations and structural rearrangements in lung, breast, prostate and pancreatic cancers, ovarian carcinoma, leukemia and melanoma (Ley et al., 2008; Mardis et al., 2009; Campbell et al., 2010; Ding et al 2010; Jones et al., 2010; Lee et al., 2010; Ley et al., 2010; Pleasance et al., 2010). Sequencing of whole transcriptome (RNA-seq) is highly sensitive and efficient method for detecting gene fusions, somatic mutations and alternatively spliced forms (Stephens et al., 2009). For example, a comparison of fluorouracil-resistant and -nonresistant human colorectal cancer cell lines revealed a global disruption of splicing in the fluorouracil-resistant cells, which was characterized by the expression of new mRNA isoforms resulting from exon skipping, alternative splice site usage and intron retention (Griffith et al., 2010). Novel somatic mutations have been

discovered by RNA-seq in granulose cell tumors of the ovary and endometriosis-associated ovarian carcinomas (Shah et al., 2009; Wiegand et al., 2010). Paired-end RNA-seq has been mainly used to comprehensively elucidate gene fusion products in cancer transcriptomes (Maher et al., 2009; Zhao et al., 2009). These preliminary studies have led to the comprehensive discovery of novel alterations in the cancer genome and new insights into the pathogenesis of cancer.

Prognostic and Predictive Molecular Signatures

For centuries its well known that medication to a given disease yields different and varied response in different patients. Even there are cases where there is no response at all. Hence, the question is: Why is this variation in response seen to the same standardized and medically approved treatment strategies? One of the recent studies has demonstrated that miRNA targeting can be used to review the clinical efficacy and resistant mechanism of various cancers (Zhou et al., 2014). Now in the genomic era, we know that the probable cause is the difference in the genomic makeup of different individuals which may be responsible for a wide range of treatment response. With the completion of the Human Genome project and the development of molecular biology techniques, genomic tests now exist to answer some of these questions. Subsequently, number of potential therapeutic targets involved in disease driving molecular pathways was unveiled (Figure 1), drastically altering the clinical evaluation and treatment of patients. By examining the genomic differences between individuals, we can understand the impact of diagnosis, prognosis and treatment. The expanding knowledge of the molecular basis of cancer has shown that variation in gene expression patterns can guide therapy not only for treating neoplastic condition but can prognostic and predictive markers.

Several published clinical practice guidelines by the National Comprehensive Cancer Network (NCCN), US

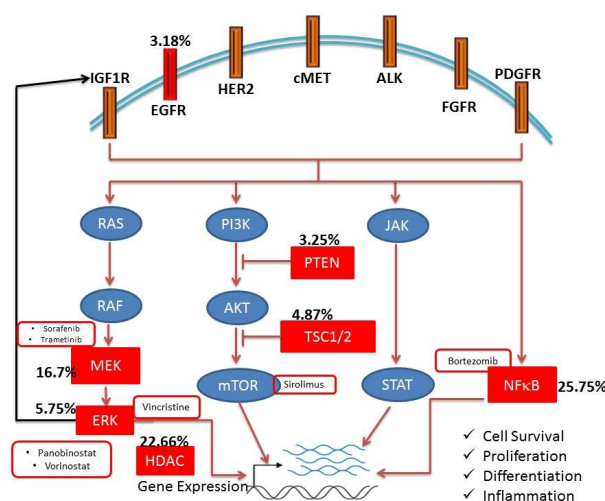


Figure 1. Showing Number of Potential Therapeutic Targets Involved in Disease Driving Molecular Pathways

FDA and the American Society of Clinical Oncology (ASCO), now recommend that all patients with NSCLC with adenocarcinoma component undergo testing for EGFR mutations and ALK rearrangements (Keedy et al., 2011; Lindeman et al., 2013) NCCN (Version 1.2015). The identification and characterization of molecular targets are having a growing impact on the management of patients with lung cancer.

Prognostic and predictive molecular signatures in surgically resected lung cancer have been developed by several research groups (Zhu et al., 2010; Kratz et al., 2013; Tang et al 2013). However, the reproducibility, cost, and lack of validation of these molecular signatures often hinder the clinical application. Zhu et al. (2010) developed a 15-gene expression panel that showed stage IB/II NSCLC patients are most likely to benefit from adjuvant chemotherapy with cisplatin/vinorelbine. Similarly, Kratz et al. (2013) developed a prognostic gene signature that was able to identify patients with early-stage NSCLC at high risk for mortality after surgical resection. More recently, Tang et al. (2013) developed an 18-gene prognostic panel in resectable NSCLC, which was then incorporated with genome-wide functional data and genetic aberration data to develop a 12-gene predictive panel for survival benefits with adjuvant chemotherapy .

Driver Oncogenic Genes and their role in Personalized Treatment

Few examples of driver oncogenes genes in lung cancer were considered if it is genetically activated in lung cancer and if there is clinically approved inhibitor or convincing proof-of-concept data that suggests high response rates in a targeted population.

EGFR

Mutations of the EGFR gene are a well-established example of an oncogenic driver in NSCLC subtype of lung cancer. EGFR activating mutations are found in ~10% and ~40% of NSCLCs in Caucasians and Asian patients respectively, and are primarily seen in adenocarcinomas (Sos et al., 2009). Several platforms are used to study EGFR mutations from tumour tissue specimens. Gefitinib, EGFR inhibitor is also approved as monotherapy for EGFR mutation-positive NSCLC following failure of platinum- and docetaxel-based chemotherapy. Another EGFR TKI afatinib recently gained FDA approval as first-line therapy for EGFR mutation-positive NSCLC. Similarly, dacomitinib-EGFR TKI, demonstrated preclinical efficacy in NSCLC tumours harbouring the T790M gatekeeper mutation (Engelman et al., 2007; Gonzales et al., 2008), found in ~50% of NSCLCs that have acquired resistance to erlotinib or gefitinib (Kobayashi et al., 2005; Pao et al., 2005).

ALK

ALK is a transmembrane tyrosine-kinase receptor expressed in the small intestine, testes, and brain, but not normally in the lung. Recently ALK gene rearrangements

have been identified as oncogenic drivers in NSCLC subtype of lung cancer. ALK signaling is activated by the creation of oncogenic fusions of the ALK gene with EML4 (Soda et al., 2007), although other fusion partners exist . ALK-EML4 rearrangements are found in ~7% of NSCLC patients (Koivunen et al., 2008; Kwak et al., 2010), usually in young never-smokers with adenocarcinoma (Rodig et al., 2009; Shaw et al., 2009; 2011; Sasaki et al., 2010). Tumours with ALK gene rearrangements are resistant to the EGFR TKIs gefitinib and erlotinib (Shaw et al., 2009).

ROS1

ROS1 is a tyrosine-kinase receptor of the insulin receptor family. ROS1 gene rearrangements are well known oncogenic drivers in NSCLC subtype of lung cancer, and several fusion patterns have been identified (Bergethon et al., 2012; Rimkunas et al., 2012). ROS1 fusions are often seen in young never-smokers with adenocarcinoma, a population similar to those with ALK-rearranged NSCLC (Bergethon et al., 2012). ROS1 rearrangements rarely present simultaneously with EGFR, ALK or KRAS alterations (Gainor et al., 2013).

Crizotinib has shown nearly complete response in a patient with advanced ROS1-positive NSCLC in clinical trial (Bergethon et al., 2012). In an expansion cohort of the trial, 14 patients received crizotinib for ROS1-rearranged NSCLC and nine (64%) had a confirmed response (Shaw et al., 2012). A further case of a complete metabolic response to crizotinib was reported in a patient with advanced ROS1-positive NSCLC . A ROS1 monoclonal antibody (D4D6) has recently been developed and validated for use in IHC assays (Rimkunas et al., 2012).

PTEN

Deleterious mutation of PTEN and loss of PTEN protein expression are common in lung cancer (Marsit et al., 2005) which plays a significant role in cell cycle progression, apoptosis, proliferation, and migration via negative regulation of the PI3K/Akt/mTOR pathway (Abdulkareem et al., 2013). PTEN loss of function mutation has also been linked with acquired resistance to EGFR TKIs in EGFR mutation-positive NSCLC (Sos et al., 2009). The TKI vandetanib has shown efficacy against EGFR mutation-positive lung cancer showing PTEN deleterious mutation, suggesting that it may also be effective in patients with EGFR mutation-positive NSCLC whose tumours lack PTEN expression (Takeda et al., 2013). However, in many cases, the functional consequences of PTEN mutations remain to be elucidated.

PI3K

PI3Ks are lipid kinases involved in the regulation of cell proliferation, growth, and survival. Deleterious mutations in the PIK3CA gene that encodes subunit of PI3K α have been identified in several cancers (Samuels et al., 2004). Additionally, aberrant signaling via PI3K/Akt/mTOR pathway has been observed in a number of human cancers, including lung cancer (Trigka et al .,2013).

PIK3CA mutations often co-exist with other oncogenic mutations, particularly EGFR, ALK, KRAS (Chaft et al., 2012). In addition, PI3K dependency in some tumours, is seen due to PTEN loss of function (Pfeifer et al., 2013). Several PI3K inhibitors are in clinical trials, but the response rate to single agents has to be yet (Pao et al., 2011; Thomas et al., 2013).

Conclusions

A deeper understanding of the molecular classification of lung cancer may ultimately lead to personalized treatment strategies, which will improve care for those patients most likely to benefit, and spare the cost and morbidity associated with failed treatment interventions. Multiplex PCR assays, high-throughput technologies such as NGS, and hopefully some form of multiplex protein-based platform will play an important role in lung carcinoma management and rational therapy selection, but there are many challenges ahead. Careful design of clinical trials will help to evaluate molecularly targeted agents in the context of those populations most likely to benefit, but clinicians will be faced with difficult decisions, such as how to include an ethically fair control arm, what treatment to choose when a new patient subset is no longer part of the first-line population, and what the preferential order of treatment should be where multiple molecular targets are present. Only through a better understanding of the disease can treatment choices be enhanced and the outlook for patients with lung cancer improved.

Although previous studies have comprehensively evaluated lung cancer at molecular level by RNA-seq, to our best knowledge none of these studies were performed with Asian population. While RNA-seq permits the simultaneous analysis of gene expression, noncoding RNA (ncRNA) expression, alternative splicing, somatic mutations and gene fusions, no systematic analyses of lung cancer transcriptomic data have been reported. RNA-seq simultaneously reveals multiple aspects of the transcriptome, including gene fusions, alternative splicing, the expression of long ncRNAs and genes, and somatic mutations.

Furthermore, characterize the global transcriptional changes in lung cancer samples by comparing them with normal, and therefore, it will be a new revolutionary direction in the pathogenesis of lung cancer biology as it would help more efficient diagnosis and drastically enhance the specificity of treatment. This study will unravel a complex landscape of functional genomic alterations in lung cancer, which will delineate concept of lung cancer heterogeneity and advance our understanding of this disease in the Indian population. Development of molecular signatures for lung cancer diagnosis if successful can prove to be path-breaking in uplifting the diagnosis and therapeutic sector of lung cancer.

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