Cancer Treatment Communications (2015) 4, 23–33

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/ctrc

Genomic aberrations guiding treatment of non-small cell lung cancer patients

Ali Saber[1,a], Anthonie van der Wekken[b,1], Thijo J.N. Hiltermann[b], Klaas Kok[c], Anke van den Berg[a], Harry J.M. Groen[b,*]

[a]*Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, The Netherlands*
[b]*Department of Pulmonary Diseases, University of Groningen, University Medical Center Groningen, P.O. Box 30.0001, 9700 RB Groningen, The Netherlands*
[c]*Department of Genetics, University of Groningen, University Medical Center Groningen, The Netherlands*

Received 28 January 2015; received in revised form 21 March 2015; accepted 28 March 2015

KEYWORDS
Non-small cell lung cancer;
Next generation sequencing;
Targeted therapy;
Resistance

Abstract
Lung cancer is the main cause of cancer-related death worldwide and conventional treatment strategies must be improved. In addition to mutations in several well-known cancer-associated genes, recent advances in sequencing technology have led to the discovery of numerous novel gene mutations and translocations. Some of these genomic aberrations occur at similar frequencies in all lung cancer subtypes, whereas others appear to be specific for adenocarcinoma or squamous cell lung cancer. High frequency mutations or recurrent translocations support involvement of the affected genes in the pathogenesis of lung cancer. The presence of activating aberrations is indicative for putative driver genes that might be essential for tumor cell growth and survival. These driver genes are potential targets for developing new treatments for lung cancer patients. Indeed, multiple tyrosine kinase inhibitors (TKIs) are currently used to treat lung cancer patients based on the presence of activating mutations, and novel drugs are under investigation. Patients benefit for about one year from current targeted treatments, but progression of disease inevitably occurs and resistance of the tumor to the TKI used can be observed in re-biopsied tumor samples. The aim of this review is to provide an overview of mutated genes in non-small cell lung cancer, an overview of targeted treatment strategies that are currently applied, and the known resistance mechanisms.

1. Lung cancer

Lung cancer is the leading cause of cancer-related deaths worldwide, with over 228,000 new cases and more than 159,000 deaths reported in 2013 in the United States [1,2].

*Corresponding author. Tel.: +31 503616161; fax: +31 503619320.
E-mail address: h.j.m.groen@umcg.nl (H.J.M. Groen).
[1]These authors contributed equally in this manuscript.

http://dx.doi.org/10.1016/j.ctrc.2015.03.005
2213-0896/

Overall, the 5-year survival rate is about 16% and late diagnosis is significantly associated with poor prognosis [1]. Lung cancer can be divided into two main subtypes based on histology: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Approximately 85% of lung cancer patients are diagnosed with NSCLC [3], which can be further subdivided into three main groups, i.e. adenocarcinoma (AC), squamous cell carcinoma (SQCC), and large cell carcinoma [4]. The AC subtype used to be more frequent in women and non-smokers, but nowadays it is more frequent than other histological subtypes in both men and women [3]. The diagnosis of lung cancer is made by histology/cytology of a tumor detected by imaging techniques such as computed tomography (CT) and positron emission tomography (PET) [5]. Treatment of lung cancer patients depends primarily on the performance status, stage of the disease, the presence of oligometastases and on histological type [6]. Surgery is the primary treatment for patients with stage I or II NSCLC [7], although adjuvant chemotherapy is advised by many guidelines to increase survival of the patients. In non-resectable, stage III NSCLC disease, chemoradiation is the preferred treatment [8]. Nowadays, treatment of lung cancer patients with advanced disease is guided by mutation analysis in the case of a documented tumor-driver mutation. The number of different tyrosine kinase inhibitors (TKIs) available for the treatment of non-small cell lung cancer patients is rapidly increasing due to new diagnostic developments.

In this review we give a brief overview of genes mutated in lung cancer, followed by a more in depth overview on potential therapeutic targets identified by next generation sequencing (NGS) technology. We also provide an overview of current targeted treatment approaches and the known resistance mechanisms.

2. Mutational landscape of lung cancer

Lung cancer, like other malignant neoplasms, is a result of an accumulation of different genetic alternations during life [9]. The *TP53* gene, originally described in 1979 [10], was the first tumor suppressor gene to be discovered. *TP53* is mutated in approximately 45% of NSCLC patients [11]. In 1982, a human gene with transforming activity was identified in a lung carcinoma cell line. This gene is homologous to the Kirsten Rat Sarcoma virus [12] and was referred to as *KRAS*. Mutations in *KRAS* are mostly found in codons 12, 13, and 61. They occur more frequently in patients with AC (5–40%) than in other subtypes of lung cancer, and are associated with smoking behavior [9].

Developments in sequencing technologies in recent years and the need to identify novel therapeutic targets have encouraged scientists to sequence large numbers of lung cancer samples. Entire gene families like protein kinases [13,14] or a combination of genes known to be mutated in lung cancer and other cancer types [15] have been analyzed. Analysis of 518 protein kinases in 33 primary lung tumors and cell lines revealed 188 somatic mutations in 141 genes, including genes known to have a role in lung tumorigenesis. For 21 genes, mutations were found in more than two samples. Seven of these genes had mutations in the kinase domain, including *BRAF*, *MAP2K4* and *FGFR2*. In addition, activating mutations were identified in *FGFR1* and *EPHA5* and inactivating mutations in *ATM* [13]. Analysis of 623 genes in 188 lung AC specimens by Ding et al. (2008) revealed 26 frequently mutated genes, including well-known cancer related genes such as *TP53*, *RB1*, *EGFR* and *KRAS* [15]. In addition, they also identified mutations in oncogenes such as *ERBB4* (*HER4*), *ERBB2* (*HER2*) and in multiple ephrin receptors (EPHAs). Altogether they observed a significant excess of mutations and copy number changes in genes involved in the mTOR, MAPK, Wnt, and the p53 signaling pathways [15]. Mutation analysis of the coding regions of more than 1500 genes of 134 primary lung tumors revealed that 18 and 19 genes were mutated at a frequency significantly above the background mutation rate in AC and SQCC, respectively. Five of these genes including *TP53*, *KRAS*, *KEAP1*, *MUC16*, and *BAI3* were shared between AC and SQCC. Differences in the set of mutated genes for various subtypes suggest that different mechanisms are involved in tumorigenesis [16]. Targeted sequencing of 145 cancer-related genes in 24 NSCLC biopsy samples, by Lipson and colleagues in 2012, revealed recurrent mutations in 21 genes, including well-known lung cancer genes together with mutations in druggable lung cancer genes such as *BRAF* and *EGFR* [17].

Together, these initial targeted and high throughput approaches indicated several targets, such as *EGFR*, *KRAS*, *BRAF* and *EML4-ALK*, that are nowadays treated with selected targeted drugs in the clinic. Although only a small proportion of all NSCLC patients (approximately 7%) will profit from these treatments (patients with complete and partial response), several tens of thousands of patients can still benefit worldwide because about 25% of patients with the subtype histology AC are suitable for studies with targeted therapies.

2.1. Potential therapeutic targets identified by next generation sequencing

Whole genome and exome sequencing (WGS and WES, respectively) have enabled researchers to dig even deeper into the mutational landscape of different cancer subtypes. These developments led to increased output of sequencing studies [18]. NGS gives us the opportunity to generate large amount of sequencing data within limited time period in a more cost effective way compared to conventional sequencing. Although, NGS is being improved every day, still we need to be careful in data interpretation and mutation calling. For instance, artifacts that can occur during sample preparation, amplification bias and DNA polymerase error should be always taken into account while working with NGS data [19].

A comprehensive overview of mutation frequencies per gene for all types of cancer is given in the COSMIC database (⟨http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/⟩). For lung cancer, the top-20 most commonly mutated genes are shown in Fig. 1.

The first studies on lung cancer using massively parallel sequencing have been performed on either cell lines or single primary tumors [20-22]. A complete genomic analysis of a single NSCLC primary tumor revealed more than 50,000 somatic variations, including new mutations in genes known