50 Gene Panel Testing in Non-Small Cell Lung Cancer and Options for Targeted Therapy

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Abstract

Lung cancer is a common cause of cancer death. Progression free and overall survival is challenging to predict due to the variety of underlying genetic defects leading to cancer development. A number of gene mutations and subsequent downstream pathway alterations have been identified in oncogenesis of non-small cell lung cancer (NSCLC), and specific genes like EGFR, KRAS, ALK, TP53 and genetic signaling pathways like PI3K/ALT/mTOR have been found to affect the prognosis and allow for options for targeted therapy. Moreover, there is evidence of high incidence of changes in receptors leading to resistance of the target sites to the available drugs causing deleterious effects on prognosis. Herein, we reviewed the available methods of diagnosing these changes at a molecular level and the implications diagnosing these alterations on survival. Literature suggests that singling out causative factors can be of prognostic value as well as serve as specific targets for therapy. Currently, a number of targeted therapies are under development. However, knowledge of genetic information opens opportunities to provide patients with a more accurate prognosis, specific treatment options, and the potential for improved treatment modalities.

Introduction

Lung cancer contributes heavily to cancer-related deaths in both men and women in the United States [1]. One of the major limitations of traditional chemotherapy and radiation is the balance between effectiveness of the treatment and its deleterious effects on normal cells and thus the patient [2]. With advancement in lung cancer genetics, the World Health Organization (WHO) classification of lung cancer has been updated to reflect genetic and molecular mechanisms causing cancer [3,4]. Lung cancer is divided primarily into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). While SCLC are associated with RB1 and TP53 mutations, NSCLC is associated with mutations in the KRAS, EGFR and TP53 genes [5].

Mutations or copy number alterations in a given sample of tissue can be detected using targeted gene panels. These panels are designed to detect genetic abnormalities that are known to be most frequently associated with the cancer in question [6,7]. Next generation sequencing (NGS) is a cohort of technologies that can be used to sequence DNA templates [8]. The sample of tissue is analyzed using fluorescence in situ hybridization (FISH) and then tested against 50 key cancer-related genes. The mutations are classified into 4 tiers: tier 1 represents variants which have an approved course of action clinically, tier 2 denotes variants that do not have an approved course of action but can be used to help with clinical decision making, tier 3 includes variants that have been previously described in malignancy and tier 4 are variants that have not yet been described in malignancy [9]. A recent study conducted by Cheng et al. used NGS on almost 150 NSCLC tissue samples for mutations or single nucleotide variants (SNV) in BRAF, EGFR, HRAS, IDH1, JAK2, KIT, KRAS, MET, NRAS and PIK3CA genes. They found that 36% of all patients who were staged IB-III had at least one of these mutations. [10] This identification of a significant number of actionable gene alterations provides options for specific treatment in patients with NSCLC [11].
Specific Genetic Mutations

Tumor Protein 53 (TP53)

The TP53 gene encodes for the p53 protein, a well-known tumor suppressor that is known as the “guardian of the genome” due to its wide antiproliferative effects. p53 is usually latent and does not participate in the progression of a normal cell cycle. Damage to DNA, oncogene activation or hypoxia activates p53 transcription which brings about downstream effects like apoptosis, cell-cycle arrest and autophagy [32-34]. It is found to be the single most frequent gene involved with multiple cancers and many of the most aggressive cancers including NSCLC have a mutated TP53 in as many as 80% of tumors, and p53 mutation have been found to correlate with poor prognosis [35,36]. Traditionally, p53 mutants have not been drug-targetable. However, there have been recent developments of compounds such as p53 reactivation and induction of massive apoptosis-1 (PRIMA-1), APR-246 (methylated derivative of PRISMA-1), PK11007 (2-sulfonylpyramidine), PK7088 (pyrazoles) zinc mettalochaperone-1 (third generation thiosemicarbazone) and peptides that are being developed with an expectation that they will be able to convert reactive properties of mutant p53 into wild type properties. However, they are still under trial for their effectiveness in humans [35].

Epidermal Growth Factor Receptor (EGFR)

Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein causing auto-phosphorylation by intrinsic tyrosine kinase activity [12]. Studies show that NSCLC with EGFR mutations are associated with higher mortality [13-15]. Epidermal growth factor binds to EGFR bringing about conformational changes and phosphorylation of the intracellular domain. This leads to downstream activation of various pathways including PI3K/Akt, Raf–extracellular signal–regulated kinase, and activation of signal transducer and activator of transcription (STAT) factors. Activation of these pathways leads to either cell proliferation or apoptosis in normal cells; mutations in EGFR can thus be oncogenic and result in abnormal cell proliferation or ability of the cell to avoid apoptosis [12,16]. Mutations in the gene coding for EGFR can be detected by polymerase chain reaction (PCR). In NSCLC, alterations in the activity of genes coding for EGFR have been reported in 43-89% of cases [17-19]. Besides mutations, amplification of gene expression can also lead to similar effects in EGFR. FISH or chromogenic in situ hybridization (CISH) are used to detect gene amplification [12].

Gefitinib and Erlotinib are two drugs that inhibit EGFR and have been recently approved for the treatment of NSCLC. They were found to inhibit the intracellular tyrosine kinase domain of the receptor [12]. Gefitinib and erlotinib provide a higher progression free survival period; however, there is no significant advantage in the overall survival when compared to chemotherapy [20]. In a recent study conducted by Zhang et al., they found that while the correlation between EGFR mutation and smoking was still controversial, the progression free survival with drugs that inhibit EGFR tyrosine kinase in patients with advanced NSCLC was better in non-smokers than in heavy smokers [22]. Peripheral blood or plasma can be used to test the EGFR mutations non-invasively, with a specificity of 88.5% and sensitivity of 64.5% by pooling data from thirteen articles [23].
Rearrangement of the ALK gene can lead to NSCLC via expression of an oncogenic fusion protein. In normal cell metabolism, the ALK gene is associated with regulating oncogenic receptor tyrosine kinases like RAS-mitogen activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/AKT and JAK-STAT pathways. Mutations cause gene partners like EML4 and NPM fusion with the intracellular domain of the receptor. As a result, abnormal proteins are expressed which promote atypical activity in the downstream signaling pathways, leading to uncontrolled cell growth and survival [37]. Shaw et al. found that 1 out of 3 patients who showed clinical characteristics similar to EGFR mutation despite having a negative EGFR mutation in fact had EML4-ALK mutations [38]. ALK mutations are estimated to be associated with 4-7% of all NSCLC and have poorer diagnosis than that observed in NSCLC overall [39]. Drugs like crizotinib and ceritinib have been developed to act on the ALK mutation target, although the development of tumor resistance to these therapies has limited widespread use [39].

Anaplastic Lymphoma Kinase (ALK)

Kirsten Rat Sarcoma viral oncogene homolog (KRAS)

KRAS is a member of the Ras family of proteins, which are small GTPases that participate in cell survival, cell cycle progression, cell structure and polarity [needs citation]. These are regulated by guanine nucleotide exchange factors (GEFs) and inhibitory GTP-ase activating proteins (GAPs) [28]. KRAS mutations are most commonly seen in patients with a significant smoking history [29]. Mutations cause RAS proteins to be insensitive to GAPs, which leads to persistent activation of downstream pathways and results in abnormal cell growth, proliferation and survival [28]. Pao et al. observed that tumors with KRAS mutations of exon 2 lacked response to kinase inhibitor drugs [29]. KRAS mutation is a poor prognostic indicator in NSCLC due to aggressive behavior and escape from targeted therapy [30,31]. As opposed to previously assumed theory that EGFR and KRAS mutations could be caused together, Gao et al. found that EGFR and KRAS mutations are in fact mutually exclusive [31].

Phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (PI3K/AKT/mTOR)

The PI3K/AKT/mTOR pathway is a prototypical oncogenic pathway in which signaling can be activated by a number of factors including upstream mutations in EGFR and KRAS. Two to five percent of all NSCLC patients had a mutation in the PI3K/AKT/mTOR pathway. Interestingly, downstream activation of this pathway plays a significant role in the acquired resistance and escape from treatment in therapies targeting EGFR mutations.
Multiple compounds have been developed to target different components of this pathway. GDC 0941, BKM 120, BAY80-6946, and PX866 are potent PI3K inhibitors, whereas GDC0068 and GSK1241795 MEK1/2 inhibit different isoforms of AKT. Everolimus, temsirolimus and ridaforolimus bind to FKBP12 protein and attenuate the downstream effects of mTOR [40].

**SRC Family of Kinases**

The SRC family of kinases (SFKs) consists of nine tyrosine-kinases and function to regulate progression of carcinogenesis. They affect pathways which are associated with the development of metastatic tumor potential. Abnormal expression of this family of genes has been implicated in various cancers, particularly human epithelial cancers. c-SRC has been found to have a special correlation to the EGFR gene. Other than NSCLC, SRC gene mutation has also been found to be contributory in breast cancers. EGFR association causes c-SRC to become activated through various mechanisms such as Tyr845 phosphorylation leading to activation of multiple downstream factors including EGFR itself. There is a strong association between SRC and EGF such that tumors having EGFR activity show increased SRC activity while inhibition of c-SRC leads to apoptosis by decreased expression of downstream signaling. While Zhang et al. found SFK activation to be associated primarily with NSCLC, Masaki et al. found the incidence is higher in adenocarcinomas. There was also evidence that depletion of c-SRC form cells reduces sensitivity to the effects of compounds like gefitinib [41,42].

**BRAF Mutations**

BRAF is a serine/threonine protein kinase that plays a relevant role in the mitogen activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway, involved in cell growth, proliferation, survival and differentiation. MAPK/ERK pathway includes several proteins with kinase domains (RAF, MEK, ERK) that convey signal transduction from membrane receptors to DNA in the nucleus of the cell. The MAPK/ERK signaling pathway is evolutionarily conserved and critical in regulating cell growth, proliferation, differentiation, migration, and survival [43]. An estimated 2.6% of NSCLCs have BRAF mutations [44]. This effort has led to the development and Food and Drug Administration (FDA) approval of several small molecule inhibitors targeting mitogen-activated protein kinase/extracellular-signal regulated kinase (MAPK/ERK) pathway tyrosine kinase proteins such as BRAF and MEK [45].

**Clinical Relevance of Gene Panel Testing**

When tumor tissue is not available for sequencing, cell-free DNA can be for next generation sequencing. However, in this context, the rate of mutated cells is less than 1% so NGS should report only clinically specific and relevant genes. 50 gene panel have been developed while taking this into consideration [46]. 

Yamamoto et al. compared sensitivity and efficiency of NGS analyses with conventional analytical methods like PCR. They found the RNA-based NGS method to be clinically convenient and effective. They were able to use solid samples from biopsies as well as cytologic samples from bronchoscopic lavage and effusion draw.
NGS provided high sensitivity over a shorter duration (4 days) at a lower expense (US$144) per sample than conventional diagnostic methods. While the conventional method diagnosed 6 mutations including 6 types of EGFR mutation and 1 type of EML4-ALK mutation, NGS analyzed exon 15-23 of EGFR mutation, all CD regions of KRAS mutations and five kinds of ALK fusion mutations [47,48].

Mayo clinic recommends the following 50 genes in a gene panel to achieve the above aims:

<table>
<thead>
<tr>
<th>Classification</th>
<th>Gene name</th>
<th>Full form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tier 1</td>
<td>EGFR</td>
<td>Epidermal Growth Factor</td>
</tr>
<tr>
<td></td>
<td>ALK</td>
<td>Anaplastic Lymphoma Kinase</td>
</tr>
<tr>
<td>Tier 2</td>
<td>KRAS</td>
<td>Kirsten Rat Sarcoma viral oncogene homolog</td>
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<tr>
<td>Tier 3</td>
<td>TP53</td>
<td>Tumor protein 53</td>
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**Conclusion**

NSCLC heavily contributes to cancer related mortality. Next generation sequencing allows for detailed knowledge of the underlying biology of each tumor, allowing for targeted therapy and the potential for improved treatments. Newer techniques of determining the underlying abnormality like NGS confer higher sensitivity in a shorter duration of time while maintaining cost-effectiveness. Additionally, this information also provides better and more specific guidelines to determine prognosis of the disease.

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