Importance of Anaplastic Lymphoma Kinase Gene Re-arrangements on Non-Small Cell Lung Cancer

Küçük Hücreli Dışı Akciğer Kanserinde Anaplastik Lenfoma Kinaz Gen Yeniden Düzenlemelerinin Önemi

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ABSTRACT
Despite all improvements in treatment modalities, lung cancer is the leading cause of death related to cancers worldwide. Environmental, occupational and genetic factors, as well as smoking play role in the etiology. Lung cancers are generally divided into two main histologic categories: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Several gene aberrations are detected in NSCLC, which constitute approximately 85% of all lung cancers. The Anaplastic lymphoma kinase (ALK) gene is a member of insulin receptor tyrosine kinase super family. ALK gene involves with translocation those results in formation of fusion protein in various malignancies such as lung cancer and lymphoma. In this article, latest literature regarding re-arrangement of ALK seen in NSCLC will be reviewed.

Key words: lung cancer; non-small cell lung cancer; ALK gene; gene rearrangements; EML4/ALK fusion

ÖZET

Anahtar kelimeler: akciğer kanseri; küçük hücreli dışı akciğer kanseri; ALK geni; gen yeniden düzenleme; EML4/ALK füzyon

Introduction
Lung cancer is an important public health issue that is leading cause of deaths related to cancer. Lung cancer is a complex disease that develops with interaction of different etiologic factors. Smoking is the most important risk factor, however environmental, occupational exposure and genetic factors also have a role. Lung cancers are divided into two main histologic types as small cell lung cancer (SCLC) (15%) and non-small cell lung cancer (NSCLC) (85%). Target-oriented and whole genome association studies showed that multi-step changes at expression level of different subtypes of lung cancer affects different oncogenic pathways. Tumoral heterogeneity that is seen in lung cancers arises from different histologic and molecular structures that can affect the treatment. Many gene aberrations are seen in NSCLC including Kirsten rat sarcoma viral oncogene homolog (KRAS), epidermal growth factor receptor (EGFR), Raf murine sarcoma viral oncogene homolog B1 (BRAF), Human epidermal growth factor receptor 2 (HER2), Proto-oncogene C-Met (MET), Anaplastic lymphoma kinase (ALK), Ret proto-oncogene (RET) gene. In this article, latest literature regarding rearrangement of ALK seen in NSCLC will be reviewed.

Non-Small Cell Lung Cancer (NSCLC)
NSCLC, that forms the majority of the lung cancers, is divided into three histologic subtypes as adenocarcinoma (AC), squamous cell carcinoma (SCC) and large cell carcinoma. AC and SCC constitute more than 70% of the cases with NSCLC. Although histologic subtypes manifest many similar biological characteristics, their cellular origins, their locations in the lungs, changes in their growth features suggest that they occur via different molecular mechanisms. Most of the patients with NSCLC are in advanced stage at the time of diagnosis.
It displays an aggressive clinical prognosis and it bears a high potential for metastasis. Therefore, overall prognosis is poor. Disease is usually in advanced stage at the time of diagnosis and 5-year survival rate is rather low (5–10%). Efficiency of agents used in chemotherapy is low. The average survival is 7.15 months in untreated advanced-stage NSCLC, while it is 8–12 months in patients who receive cytotoxic platinum-based chemotherapy. Changes in approximately 140 genes were detected in studies regarding mechanisms of carcinogenesis in previous decades. They have a “driver mutation” function on initiation and progression of malignancy. In most of adult malignancies, 33–36 driver mutation is found, and this reaches ~200 mutation in NSCLC with addition of mutagenic agents such as cigarettes.

Recently, it was found that NSCLC subtypes treated with standard chemotherapeutic agents showed different genetic changes and this in turn affects response to therapy and progression-free survival (PFS). Therefore, treatment protocols intended for histologic type have come into question. Our knowledge on initiator mutations seen in NSCLC was increased with the use of high throughput screening techniques. Detection of activating mutations in EGFR kinase domain has been particularly instructive. Improvement in response to treatment and progression-free survival was achieved with use of EGFR-tyrosine kinase inhibitors (EGFR-TKIs) compared to standard chemotherapeutic agents. Today purpose of cancer treatment is to provide individual-specific treatment with knowledge of tumor molecular changes.

**Anaplastic Lymphoma Kinase (ALK) Gene**

ALK is localized in 2p23.2 and consists of 29 exons. This gene, a member of insulin receptor protein-tyrosine kinase super family, codes a 220 kDa protein. ALK gene was first identified by Morris et al. in 1994 as a result of translocation made with nucleophosmin on large cell lymphoma cell line t(2;5)(p23;q35). This chromosomal rearrangement results in ectopic expression of NPM-ALK fusion protein that activates kinase domain of the ALK gene.

The ALK protein consists of three main parts including an extracellular ligand-binding domain, a transmembrane domain and a single intracellular tyrosine kinase domain. Tyrosine residues are phosphorylated following activation of ALK through dimerization. ALK protein acts on neuronal development and differentiation during embryogenesis in mammalians. It is reported that ALK mRNA is expressed in human brain, testis, prostate and colon, however it is not expressed in spleen, lungs, ovaries, placenta, liver, skeletal muscle, kidneys, lymphoid tissue and pancreas. Active ALK Janus kinase, which is a mammalian target of rapamycin, induces several signal transmission pathways such as sonic hedgehog, hypoxia-inducible factor-1α, JUNB, and phospholipase signaling.

ALK gene activation occurs through three distinct mechanisms involving i) fusion protein formation, ii) ALK overexpression, and iii) activating ALK point mutations. While one of these mutations is observed in histologically identified cancers, two types of mutations can be seen in NSCLC. Fusion partner in ALK translocations regulates the level of ALK expression, cellular location and time of its expression. Intracellular kinase domain of ALK and terminal end points of different genes make up the fusion protein.

Twenty-two distinct translocation partners that are known to form fusion protein with ALK exist. In lung cancer, fusion with Echinoderm microtubule-associated protein-like 4 (EML4) gene was detected first and TFG-ALK, KIF5-ALK fusions followed. ALK gene mutations are seen in several cancers including anaplastic large cell lymphoma, diffuse large B cell lymphoma, neuroblastoma, inflammatory myofibroblastic tumor, lobular breast cancer, colorectal cancer, renal cell carcinoma, esophageal squamous cell carcinoma and NSCLC adenocarcinoma.

**ALK Gene in NSCLC**

ALK-EML4 rearrangement in NSCL was defined in Japanese patients in 2007 by Soda et al. Both EML4 and ALK gene are located on the short arm of the chromosome 2, and fusion formation occurs with the inversion in p arm of the chromosome 2, N-terminal domain of the EML4 and intracellular kinase domain of the ALK gene forms a fusion, and a continuously active ALK tyrosine kinase effect arises. EML4-ALK fusion activates the downstream RAS/RAF/MEK, PI3K/AKT/mTOR and Janus kinase (JAK)/STAT signal pathways that will lead to cellular proliferation, invasion and inhibition of apoptosis.

Multiple variants of EML4-ALK have been reported (Table 1). They encode the same intracellular tyrosine kinase domain of ALK, but different truncations of EML4. The most common variants are variant 1 (detected in 33% of NSCLC patients) which results in the juxtaposition of exon 13 of EML4 to exon 20 of ALK (E13; A20) and variant 3a/b (29% of NSCLC...
patients) in which exon 6 of EML4 is integrated into exon 20 of ALK (E6a/b; A20)\textsuperscript{10}.

Diverse variants of EML4-ALK proteins can manifest different enzymatic activity. ALK also has multiple fusion partner that display different molecular weight, protein stability and ALK inhibitor sensitivity\textsuperscript{4}. In monkey models, it was found that EML4-ALK fusion gene showed oncogenic activity\textsuperscript{9}. EML4-ALK appears rare in lung cancer and has been detected in about only 3–8\% of NSCLC cases\textsuperscript{11}. EML4-ALK fusion bears distinct pathological and demographic characteristics. One of the most striking features of EML4-ALK-positive lung cancers is early age (~50) of onset\textsuperscript{12}.

EML4-ALK fusion in NSCLC is more common in adenocarcinomas and non-smokers or light-smokers\textsuperscript{12}. Preclinical and clinical studies showed that cancer cells with EML4-ALK or other ALK anomalies are rather sensitive to ALK inhibitor drugs\textsuperscript{13}.

**ALK Testing in NSCLC**

In order to detect ALK rearrangement in NSCLC, methods such as FISH, IHC and reverse transcriptase RT-PCR are used\textsuperscript{1,14,15}. Every method has its own advantages and limitations\textsuperscript{16}. Today, FISH method using break-apart probes is accepted as the gold standard, and its use is approved by the food and drug administration (FDA) to identify ALK-rearranged NSCLC\textsuperscript{17}. Despite many advantages, the ALK FISH test has also several limitations. It requires well-established laboratories with an experienced operator, and its cost is high. IHC is an easier and less expensive method, usually available in local laboratories, based on the use of ALK-specific monoclonal antibody.

Many studies suggested a marked correlation between ALK-rearrangement positivity, as detected by FISH, and ALK protein overexpression, as detected by IHC. These findings imply that IHC could be used for screening of ALK rearrangements prior to FISH, leading to the development of new diagnostic algorithms, and this must be validated in large scale concordance studies. In conclusion, RT-PCR is the most sensitive method of detecting not only ALK re-arrangements, but also of determining their variant types, and the abundance of EML4-ALK positive cells in NSCLC tumor tissue. Its another advantage is requirement of limited amount of material for analysis and it is rather easy to perform, however the development of this method as a diagnostic tool has several limitations\textsuperscript{16}.

**ALK Targeted Treatment in NSCLC**

1. **First generation of ALK inhibitors:**

   Crizotinib, an oral small-molecule tyrosine kinase inhibitor (TKI) of ALK, MET and ROS1 kinases, is a first-generation ALK inhibitor\textsuperscript{18}. In 2011, US FDA approved crizotinib for treating ALK-positive NSCLC. It was also approved by the European Committee for Medicinal Products for Human Use in 2015 for NSCLC patients as the standard first-line treatment. Crizotinib has a longer PFS and approximately 53–65\% better objective response rate (ORR) compared to chemotherapy\textsuperscript{19}. It was reported that patients acquire drug resistance within the first year following the initial use of crizotinib\textsuperscript{20}.

   The mechanisms of crizotinib resistance were suggested to involve point mutations, fusion gene amplification, and activation of bypass signaling via activation of other oncogenes including EGFR, MAP kinase-ERK kinase (MEK), extracellular signal regulated kinase (ERK), SRC proto-oncogene (SRC) and insulin-like growth factor-1 receptor (IGF-1R)\textsuperscript{21}. Particularly, there were two mechanisms noticed in about 33\% of patients who developed the secondary point mutation after treatment with crizotinib. Several point mutations were detected including G1269A, F1174L, L1152R, S1206Y, 1151Tins, I1171T, D1203N, V1180L, C1156Y, F1164V, G1202R, G1269S\textsuperscript{22}. The most common of these mutations are L1196M and G1269A\textsuperscript{23}.

2. **Second generation of ALK inhibitors:**

   The purpose of second-generation ALK inhibitors was to refrain from the CNS progression caused by first-generation ALK inhibitors that occurs in approximately 70\% of the NSCLC patients who has brain metastasis\textsuperscript{24}. Besides, they were developed as a result of the need to improve PFS efficacy. Second-generation ALK inhibitors include ceritinib, alectinib, brigatinib, entrectinib, X-396 and TSR-011\textsuperscript{25}. Among these, ceritinib and alectinib were recently approved by FDA for the treatment of ALK-rearranged NSCLC. The second-generation ALK inhibitors could hinder...
the resistance mutations which occur due to the first-generation inhibitor crizotinib, and increase the potency against central nervous system diseases. Yet, treatment with the second-generation ALK inhibitors also results in drug resistance and tumor recurrence. Ceritinib was approved by FDA in 2014 for the treatment of NSCLC. This small molecule is an oral tyrosine kinase inhibitor of ALK and exhibits an ATP-competitive action.

The most common resistant mutations occurring due to ceritinib include G1202R and F1174L. C1156Y, 1151Tins and L1152R secondary mutations were also found to be related with resistance caused by ceritinib. Brigatinib is an inhibitor acting on ALK and EGFR and it was found to hinder mutations resistant to crizotinib such as ALK L1196M and the gatekeeper mutation T790M of EGFR.

3. The third generation of ALK inhibitors:
Second-generation ALK inhibitors may cause tumor recurrence and cerebral metastases. Lorlatinib is a more efficient ALK/ROS1 inhibitor and a phase 2 clinical trial has been conducted on it. There is evidence obtained from in vitro studies that Lorlatinib had better therapeutic potency compared with the secondary ALK mutations caused by crizotinib. It has been recently reported that L1198F mutation is resistant to lorlatinib primarily via steric interference with drug binding. Other studies reported that L1198F could promote the crizotinib binding, diminish the C1156Y effect, and re-sensitize resistance to crizotinib. It appears that the combination of lorlatinib and PI3K pathway inhibitors exhibit more potency in reducing ALK mutations and ALK inhibitor resistance, however clinical impacts or resistance mechanisms of lorlatinib should be further studied to overcome L1198F.

Resistance Mechanisms in ALK Targeted Treatment of NSCLC
Although crizotinib has a perfect efficacy in ALK-positive lung cancer cases, almost all patients show resistance to crizotinib sooner or later. Mechanisms of this resistance can be divided into two major groups: ALK-dominant or ALK non-dominant. The various mechanisms submitting intrinsic or acquired resistance to crizotinib are presented in Table 2.

Novel Strategies to Overcome Resistance
Two distinct strategies with ongoing phase I/II clinical studies on crizotinib resistance are new generation ALK inhibitors (e.g., AP26113, LDK378, and CH5424802) and heat shock protein 90 inhibitors (e.g., STA-9090, IPI-504, and AUY922). New generation ALK inhibitors effectively inhibit ALK kinase and show activity against most of the resistance mechanisms in in vivo/in vitro tests. HSP90 inhibitors are not specific to ALK, however they can overcome crizotinib resistance by diminishing folding in oncogenic proteins including ALK fusion proteins. In other cases with oncogenic drivers activation, ALK dual inhibition and changing enzymes take over in potential treatment strategies.

Conclusion
Accumulating information about cancer biology and oncogenic drivers has provided the scientists a better comprehension of lung cancer and introduction of efficient targeted therapies. Evidence supports that ALK inhibitors can help ALK-positive NSCLC patients, however inevitable drug resistance remains as a problem. Thanks to clear understanding of ALK-positive cancer-specific pathophysiology, better therapy for patients with lung cancer and reduced resistance can be achieved by improvement of ALK inhibitors. Gene heterogeneity, mutation number and location have a significant role in mechanisms of resistance, where the resistance of one inhibitor develops due to multiple mutations and factors, or some inhibitors sue to another factor. One of clinical difficulties is efficient and precise assessment of drug resistance to ALK inhibitors in order to improve the treatment during disease progression. Further studies will focus on optimal diagnosis and treatment at earlier stages of disease, also on rational
combinations of effective agents and the ideal treatment regime, especially as more next-generation agents are being approved. Besides, ideal supportive care and toxicity management is crucial for the cancer cases who may hopefully have a better survival on sequential treatment.

References


