

The Importance of Screening for EGFR Mutation in Lung Cancer

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ABSTRACT

Lung cancer is one of the cancers with the most cases occurring in both men and women. It was found that a number of patients with lung cancer had mutations in the epidermal growth factor receptor (EGFR). The EGFR mutation is a somatic mutation associated with non-small cell lung cancer (NSCLC), which can be identified using real-time PCR or Next Generation Sequencing (NGS) in a sample obtained from formalin-fixed paraffin-embedded tissue. The presence of EGFR mutation in lung cancer tissue has revealed an opportunity to treat this disease with anti-EGFR therapy. First-generation EGFR inhibitors work by reversibly inhibiting the EGFR tyrosine kinase. However, it does not work with the T790M mutation which needs a third-generation EGFR inhibitor that can irreversibly inhibit both active and inactive variants of EGFR. Screening of EGFR mutation in NSCLC has been mandatory to reveal an opportunity for treating with EGFR inhibitor.

Keywords: lung cancer; EGFR mutation; anti-EGFR

INTRODUCTION

Lung cancer is one of the cancers with the most cases occurring in both men and women. According to the Global Burden, lung cancer is among the top three cancer cases and the main cause of cancer death in Indonesian men and women [1]. In 2023, it is estimated that there will be about 238,340 new cases of lung cancer, with 117,550 in men and 120,790 in women. About 10% to 15% of all lung cancers are SCLC (small cell lung cancer), while about 80% to 85% are NSCLC (non-small cell lung cancer). Lung cancer is mainly diagnosed in older people, with most people diagnosed being 65 or older, and the average age of diagnosis being about 70 [2]. The prognosis of lung cancer patients is influenced by the stage of cancer experienced, the higher the stage of cancer, the worse the prognosis for life. This shows that lung cancer patients with stage 4 have higher mortality [3]. Stage 4 lung cancer patients are difficult to treat because the cancer has spread to other organs so it can only be treated to relieve the symptoms they are experiencing [4]. However, it turns out there is research that advanced lung cancer patients who have EGFR mutations can have hope of being treated as long as the type of mutation is properly detected.

Among the patients with lung cancer, it was found that there were patients who had EGFR mutations. There were 30,466 patients with EGFR mutation out of 115,815 NSCLC patients [5]. However, it turns out that in patients with stage 4 lung cancer, EGFR (Epidermal Growth Factor Receptor) mutations, anti-EGFR drugs, or EGFR tyrosine kinase inhibitors

can be given to improve life prognosis [6]. Some of the first-line anti-EGFR drugs that can be given are erlotinib, gefitinib, and osimertinib [7]. This EGFR mutation does not occur in all lung cancer patients so it is necessary to carry out an EGFR mutation test.

Epidermal Growth Factor Receptor (EGFR) Mutation

Epidermal Growth Factor Receptor (EGFR) is a receptor on the cell membrane that, when bound to its ligand, causes the cell to proliferate. However, when EGFR undergoes a mutation, it will proliferate even though it does not bind to its ligand. The presence of an EGFR mutation indicates that this cancer is more malignant.

Mutations in the EGFR (Epidermal Growth Factor Receptor) do contribute to the Ras-MAPK (Mitogen-Activated Protein Kinase) pathway in non-small cell lung cancer (NSCLC). One essential signaling cascade for cell survival, proliferation, and growth is the Ras-MAPK pathway. Mutations in this pathway's constituents, such as EGFR, can result in abnormal signaling and aid in the emergence and spread of cancer, including NSCLC [8].

The Ras-MAPK pathway in normal cells is triggered by growth hormones like epidermal growth factor (EGF). The Ras protein is activated as a result of a sequence of intracellular events that are started when EGF attaches to the EGFR. Extracellular Signal-Regulated Kinase (ERK) is one of the MAPKs that are phosphorylated and activated as a result of a kinase

cascade that is triggered by activated Ras. Once within the nucleus, the activated ERK controls the production of genes essential for cell division, proliferation, and survival [9].

EGFR mutations are genetic changes that arise in NSCLC related to the EGFR gene. Even in the absence of exogenous growth hormones like EGF, these alterations have the potential to cause constitutive activation of EGFR. Point mutations, insertions, and in-frame deletions in the kinase domain of the EGFR gene are examples of EGFR mutations [9]. L858R point mutation and exon 19 deletions are two frequent EGFR mutations in non-small cell lung cancer (NSCLC) [10].

When EGFR mutations occur in non-small cell lung cancer (NSCLC), downstream signaling pathways, such as the Ras-MAPK pathway, are persistently activated. Due to their hyperactive kinases, mutated EGFR proteins cause persistent signaling via downstream MAPKs like ERK and Ras. Prolonged activation of the Ras-MAPK pathway in non-small cell lung cancer (NSCLC) leads to uncontrolled cell proliferation, resistance to programmed cell death, and tumor development [9].

Non-small cell lung cancer (NSCLC) is primarily caused by mutations in the EGFR (epidermal growth factor receptor) protein, which activates the Ras-MAPK (mitogen-activated protein kinase) pathway. However, these mutations can also affect the PI3K (phosphoinositide 3-kinase) signaling pathway. Both routes are frequently activated simultaneously, which aids in the survival and growth of cancer cells [8].

The PI3K signaling system controls metabolism, cell viability, and growth. Growth factors, like EGF (Epidermal Growth Factor), activate downstream signaling pathways, such as PI3K, when they link to receptors like EGFR. Phosphatidylinositol 3,4,5-trisphosphate (PIP3) is produced when PI3K is activated, and PIP3 in turn stimulates protein kinase Akt (also known as Protein Kinase B or PKB). Because it phosphorylates several downstream targets, activated Akt is essential in both stimulating cell proliferation and preventing apoptosis or programmed cell death. In non-small cell lung cancer (NSCLC), EGFR mutations may cause downstream signaling pathways, such as PI3K and Ras-MAPK, to become overactive [11].

EGFR Mutation Inhibitor

Two kinds of medications, known as first- and third-generation EGFR (Epidermal Growth Factor Receptor) inhibitors, are used to treat specific cancers, most notably non-small cell lung cancer (NSCLC). The EGFR protein, which is essential for cell division and proliferation, is the target of these inhibitors. First-generation and third-generation EGFR inhibitors differ primarily in their mechanism of action, resistance profiles, and therapeutic uses [12].

First-generation EGFR inhibitors, such as gefitinib and erlotinib, work by reversibly inhibiting the EGFR tyrosine kinase. They compete with ATP to bind to the EGFR kinase domain, preventing downstream signaling and phosphorylation of the protein. They primarily target EGFR's active form [13]. On the other hand, third-generation EGFR inhibitors, such as osimertinib, target just the mutant versions of EGFR that frequently become resistant to first-generation inhibitors. These drugs bind to the EGFR kinase domain of both active and inactive variants of EGFR in an irreversible covalent manner, preventing downstream signaling and kinase activity [14].

Patients with EGFR-mutated non-small cell lung cancer (NSCLC) respond well to first-generation EGFR inhibitors; nevertheless, resistance to these medications sometimes develops over time due to EGFR mutations that occur later in life (e.g., the T790M mutation) or other factors. The majority of acquired resistance instances are caused by the T790M mutation [15]. Third-generation EGFR inhibitors have shown effective in treating patients who have become resistant to first-generation inhibitors and are intended to overcome the T790M resistance mutation. They also have a lower risk of developing resistance due to their unique binding mechanism [14,15].

Patients with non-small cell lung cancer (NSCLC) who have EGFR-activating mutations are generally treated with first-generation EGFR inhibitors as first-line treatment in the clinical setting. However, the emergence of resistance to them can restrict their efficacy and frequently cause the condition to worsen. When first-generation EGFR inhibitors are no longer effective for treating NSCLC patients because of the T790M mutation, third-generation EGFR inhibitors are typically utilized as a second-line treatment [12].

Threonine-to-Methionine (T790M) Mutation

Resistance to first-generation EGFR (Epidermal Growth Factor Receptor) inhibitors, such as erlotinib and gefitinib, in the treatment of non-small cell lung cancer (NSCLC), is often associated with specific mutations in the EGFR gene. The T790M mutation is the most prevalent resistance mutation that arises in response to first-generation EGFR inhibitors [15].

The T790M Mutation (Threonine-to-Methionine Mutation) is a point mutation in the EGFR gene. At position 790 in the EGFR protein, methionine (M) is substituted for the amino acid threonine (T) [16]. Patients with NSCLC who initially react to first-generation EGFR inhibitors are usually the ones with the T790M mutation. By altering the EGFR protein's structure to make it less vulnerable to these medications' inhibition, it increases resistance [12].

Patients with NSCLC who acquire the T790M mutation frequently see a progression in their disease despite taking first-generation EGFR therapies.

Because of this mutation, the medication may attach to the EGFR receptor less strongly, keeping the receptor active and enabling it to continue signaling for cell division and growth [14].

Therefore, identifying the T790M mutation in the EGFR (Epidermal Growth Factor Receptor) gene is crucial to determining the optimal treatment plan for patients with non-small cell lung cancer (NSCLC) who have developed resistance to first-generation EGFR inhibitors. The T790M mutation can be found by a number of molecular techniques, including fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), next-generation sequencing (NGS), real-time polymerase chain reaction (PCR), and DNA sequencing [17].

Certain mutations in the EGFR gene, such as T790M, can be identified by Sanger sequencing or DNA sequencing. It might not be as sensitive as some more recent methods, though. Using Allele-Specific PCR, the T790M mutant allele may be identified and amplified with precision. For identifying this particular mutation, it is both sensitive and specific. Targeted NGS Panels are helpful for finding different mutations, such as T790M, and are capable of sequencing numerous genes at once. They are useful for comprehending the larger genetic profile of a patient's tumor because of their great sensitivity and comprehensiveness [18].

T790M-specific antibodies against EGFR can be used to stain tissue samples immunohistochemically in order to identify the T790M mutation. IHC is a supplementary method in certain situations, although not being employed as frequently as molecular tests. On the other hand, T790M and other gene amplifications and mutations can be found in tissue samples using EGFR T790M FISH Probes. Fluorescently labeled probes that bind to particular DNA sequences are used in FISH [18].

Screening for EGFR Mutation

Both Next-Generation Sequencing (NGS) and Real-time Polymerase Chain Reaction (PCR) are useful methods for identifying EGFR (Epidermal Growth Factor Receptor) mutations in cancer patients, including mutations like T790M. The choice between the two approaches is influenced by a number of criteria, such as the particular requirements of the analysis, the resources at hand, and the clinical setting. Each method has pros and cons of its own [19].

Advantages of real-time PCR include its high sensitivity and specificity when tailored for a particular mutation. It is capable of precisely determining if the target mutation is present or absent. When it comes to speed, Real-time PCR is a somewhat quick method that can yield results in a few hours. When a prompt diagnostic or treatment choice is necessary, this may be helpful [20]. In many cases, this approach is less expensive than NGS. It works well in scenarios when sample material is scarce, like fine-needle aspirates or small biopsies, because it only needs a small amount of DNA [21].

The disadvantages of real-time PCR are not suitable for comprehensive genomic profiling or the discovery of new mutations that were not specifically targeted because this method is designed to detect specific known mutations and Real-time PCR is only designed to detect a single mutation while multiple assays are needed to detect multiple mutations, which can increase the complexity and cost of testing [22].

NGS (Next-Generation Sequencing) has the benefit of allowing for the simultaneous examination of many genes and a broad spectrum of mutations, which makes it appropriate for thorough genomic profiling. Not only can NGS detect existing mutations, but it can also find new ones. This is especially helpful for finding uncommon or newly discovered mutations in studies and clinical trials. When evaluating clonality and heterogeneity within malignancies, NGS offers quantitative information regarding the frequency of mutations within a sample [23].

However, NGS also has drawbacks, such as being often more expensive than real-time PCR because of the substantial data analysis and sequencing that go into them. Compared to real-time PCR, NGS procedures are more complicated and need specialized tools, knowledge of bioinformatics, and longer turnaround times. Larger and higher-quality DNA samples are usually needed for NGS analysis, which may not be possible from tiny or damaged samples. NGS results can be difficult to interpret, and specialist knowledge may be needed to find pertinent mutations among massive volumes of genomic data [24].

CONCLUSION

Somatic mutation of the EGFR gene has been detected in several non-small cell lung cancer (NSCLC) patients. This mutation has offered an opportunity to treat lung cancer with EGFR inhibitors. Since this mutation did not occur in every lung cancer patient, detection of EGFR mutation in NSCLC has been mandatory for response therapy prediction in treating lung cancer with EGFR inhibitors.

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