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The Resistance to EGFR-TKIs in Non-Small Cell Lung Cancer

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Abstract. Lung cancer has emerged as a highly aggressive malignancy posing a significant global health hazard, with a particular concern for the rising incidence and mortality of gene-altered non-small cell lung cancer (NSCLC). As the prevalence of NSCLC rises, the investigation and selection of tumor treatment techniques are gaining significance. The treatment of individuals with genetic mutations in advanced NSCLC has emphasized the application of epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs). Early and subsequent generations of EGFR-TKIs have demonstrated clinical efficacy in NSCLC patients carrying select genetic alterations. Osimertinib, a third-generation EGFR-TKI, has been sanctioned as the standard first-line therapy and is also utilized as a second-line option for individuals who develop the T790M resistance mutation following prior EGFR-TKI treatment. Approximately 10 to 30 percent of patients diagnosed with NSCLC exhibit EGFR mutations. This leads to abnormal activation of cancer genes and inactivation of tumor suppressor genes, resulting in poor prognosis for patients. This paper aims to analyze the development and resistance processes of EGFR inhibitors, thereby enhancing the comprehension of the resistance pathways to EGFR-TKIs.

Keywords: EGFR mutation, EGFR-TKIs, Non-Small Cell Lung Cancer.

1. Introduction

Lung cancer was one of the most prevalent kinds of cancer types in an analysis of cancer incidence and mortality rates conducted in 2022, comprising 12.4% of the total cancer diagnoses [1]. NSCLC is the most predominant subtype of lung cancer, making up about 85% of all diagnosed lung cancer instances. The predominant proportion of NSCLC diagnoses occur at a metastatic stage, with patients typically exhibiting a lower overall survival (OS) rate that is statistically unfavorable [2], due to a lack of early symptoms. Even though conventional treatments like surgery and chemotherapy work well, they still have limitations when it comes to controlling tumor recurrence and ensuring patient survival. Therefore, the ongoing investigation is focused on refining strategies for the NSCLC therapy. The epidermal growth factor receptor (EGFR) belongs to the receptor tyrosine kinase superfamily. The EGFR with genetic mutations has emerged as a primary target in the therapeutic approach of NSCLC, rendering the domain of molecular targeted therapy a substantial opportunity. Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) have significantly improved OS and progression-free survival (PFS) in patients with non-small cell lung cancer (NSCLC) relative to traditional chemotherapeutic agents, while also exhibiting fewer adverse effects. The primary representative drugs are the first-

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generation reversible EGFR-TKIs and the second-generation irreversible EGFR-TKIs. These agents demonstrate enhanced efficacy for patients with sensitive EGFR gene mutations and are frequently employed in clinical practice for the advanced NSCLC treatment. Additionally, patients with metastatic or advanced EGFR-mutated NSCLC who have developed resistance mutations to first- and second-generation EGFR-TKIs are now qualified for treatment with osimertinib, a third-generation EGFR-TKI. Although modern EGFR-TKIs can transiently impede disease progression, resistance arises in NSCLC patients due to re-mutation of the EGFR gene. Consequently, investigating resistance pathways has emerged as a prominent focus in the global research arena for NSCLC. The purpose of this article is to review the processes driving TKIs resistance and the developments in related research.

2. EGFR-TKIs in NSCLC

Gefitinib, a first-generation drug, selectively inhibits the EGFR tyrosine kinase by competitively blocking the ATP binding site within the intracellular protein tyrosine domain, thus impeding tyrosine kinase activity, and demonstrates a strong affinity for EGFR exon 19 deletions and exon 21 L858R mutations. According to recent randomized research, gefitinib improves disease-free survival (DFS) for patients with resected stage II–IIIA non-small cell lung cancer (NSCLC) with EGFR mutation-positive tumors when used as an adjuvant treatment. Cisplatin and vinorelbine are the two chemotherapy medicines in this study that show relatively low efficacy [3]. Dacomitinib, a second-generation EGFR-TKI, can covalently bind to pan-ErbB family TKI with strong affinity. Dacomitinib exhibits a stronger inhibitory effect on the proliferation of EGFR-dependent tumors compared with first-generation TKIs. An open-label, randomized Phase III study evaluated the effectiveness of Dacomitinib versus the first-generation TKI Gefitinib as a first-line therapy for individuals with EGFR-activating mutations and advanced NSCLC. In terms of comparing OS, Dacomitinib showed better results, with a 42-month OS probability of 41.0% compared to 33.6% for Gefitinib [4]. More and more recent studies highlight the potential of this generation of TKIs in targeting rare EGFR gene mutations, such as L816Q and G719X. An article reports the results of an experiment that grouped sensitized rare EGFR mutation NSCLC patients who had not received treatment. The results showed that using Afatinib as the initial treatment for rare or complex EGFR mutation-positive advanced NSCLC was superior to platinum-based dual-drug chemotherapy [5]. Despite evidence indicating the potential usefulness of second-generation TKIs, limitations persist, necessitating further experimental data to substantiate this assertion. Dacotinib's safety and therapeutic effect in treating late rare mutations are presently being studied in another clinical study, the purpose is to generate more conclusive experimental results [6]. The emergence of third-generation TKIs has effectively eliminated the T790M resistance mutation that occurs during the treatment period with the first and second generations. In a Phase III trial, using Osimertinib as adjuvant treatment significantly outperformed placebo in terms of DFS for stage IB-IIIA NSCLC patients with gene mutations following total removal of the tumor, with a lower incidence of local or distant metastasis [7]. In a clinical trial focusing on previously untreated advanced NSCLC patients, the first-line treatment of osimertinib reached an OS of 38.6 months. This duration was substantially longer than that of the control group, which received either Gefitinib or Erlotinib and had an OS of 31.8 months [8]. For patients with CNS brain metastases, osimertinib also showed greater activity and was correlated with a decreased incidence of severe adverse events.

3. Primary (Intrinsic) Resistance to EGFR-TKIs

Recently, the mechanisms of intrinsic resistance to EGFR-TKIs have garnered significant interest. Although studies have demonstrated that the majority of EGFR-TKIs are useful in treating patients with common mutations, a small group of people, possibly due to some uncommon gene mutations, are inherently resistant to EGFR-TKIs from the initial stage. Compared to acquired resistance, intrinsic resistance mechanisms may be more complex. Numerous studies have shown that exon 20 insertion may lead to intrinsic resistance to EGFR. Exon 20 insertions take place in the N-terminal lobe of the EGFR receptor, spanning from residue M766 to C775, resulting in the addition of extra amino acids. The C-helix region, encompassing amino acids A767 to C775, is the favored site for these insertions. This

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region is very important in regulating receptor activity and can maintain the activity of this kinase domain through conformational changes [9]. Additionally, this rare mutation, unlike common mutations, activates EGFR without increasing the affinity for the EGFR-TKI receptor [10]. Another potential intrinsic resistance mechanism may be related to the deletion polymorphism of BIM, a molecule belonging to the Bcl-2 family that participates in the intrinsic pathway of cell apoptosis. However, the regulatory mechanisms of BIM are not yet completely understood. One potential explanation is that polymorphism may lead to BIM subtypes lacking the pro-apoptotic BH3 domain, thereby promoting resistance to EGFR TKIs [11]. Research demonstrated that PFS and OS did not significantly differ between individuals with and without the BIM deletion polymorphism, with median PFS of 12 months compared to 11 months and median OS of 31 months vs. 30 months, respectively [12]. Further research indicates that the BIM deletion polymorphism does not correlate with primary resistance to EGFR-TKIs [13]. Therefore, additional studies are necessary to ascertain whether BIM loss polymorphism is linked to intrinsic drug resistance mechanisms. In addition, two recent different studies have also explored other potential mechanisms of intrinsic resistance to EGFR-TKIs. One study suggests that abnormal hypermethylation of 5-methylcytosine (m5C) in the ribose chain, along with the overexpression of NSUN2, is closely associated with intrinsic resistance to Gefitinib [14]. Another study found that overexpression of cyclin D1 and deletion of p16 (CDKN2A) may lead to intrinsic resistance to osimertinib [15].

4. Mechanisms of Acquired Resistance to EGFR-TKIs

The T790M mutation is recognized as the most frequent acquired resistance, constituting roughly 60% of the overall incidence of drug resistance in the treated population. This genetic alteration corresponds to a missense mutation, wherein threonine is substituted with methionine at the 790th amino acid residue within the coding sequence of exon 20. Its residue is positioned at the gateway to the hydrophobic pocket within the ATP binding region, a critical area for EGFR-TKI action on EGFR, resulting in the emergence of resistance. The methionine residue, with its larger side chain, induces conformational changes and creates steric hindrance. Additionally, the T790M mutation enhances the binding affinity between EGFR and ATP, thus reducing the ability of inhibitors to competitively block the receptor [16]. However, in an early 2016 randomized, international, open-label phase III trial, it has been elucidated that osimertinib exerts enhanced therapeutic efficacy as compared to platinum-based chemotherapy with pemetrexed in the management of patients afflicted with advanced NSCLC who have acquired the T790M resistance mutation subsequent to initial TKIs therapy, while concurrently mitigating the occurrence of treatment-related adverse effects [17]. Recently, a phase 2 study conducted by multiple hospitals across mainland China indicated that befotertinib (D-0316), an innovative third-generation EGFR-TKI, has a significant therapeutic effect in treating patients who have acquired the T790M mutation [18]. Therefore, befotertinib may provide a novel alternative for patients resistant to first- and second-generation inhibitors who possess the T790M mutation. Additionally, when using osimertinib to treat individuals with NSCLC who show positive for T790M, the most common tertiary resistance mutation that emerges is the C797S mutation. This mutation replaces cysteine with serine at codon 797 in exon 20 of the ATP binding site, preventing the covalent binding of EGFR to osimertinib and thereby leading to resistance. Another rare form of mutation (C797G) has also been reported in clinical trials. The C797S mutation can form a triple mutation with the previous two mutations, making it a hot spot for EGFR-dependent resistance mutations. Fortunately, only 7% of individuals with the C797S mutation were identified when osimertinib was used as a first-line treatment [19]. However, a 15% incidence of the C797S mutation was noted in patients undergoing osimertinib as a second-line therapy [20], indicating that the C797S mutation is predominantly associated with osimertinib utilized in this context. Notably, patients who do not have the T790M mutation but possess the C797S mutation may still maintain some sensitivity to early-generation EGFR-TKIs, making these drugs a potential treatment strategy for such cases. The concurrent presence of both mutations may enhance the efficacy of first-generation TKIs when used alongside third-generation TKIs. The distinct genetic loci of the T790M and C797S mutations can affect therapy options. When both mutations coexist, the existing EGFR-TKIs, whether administered singly

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or in conjunction, may not demonstrate efficacy. Should the T790M and C797S mutations reside on separate alleles (in trans), the amalgamation of first- and third-generation TKIs may demonstrate an anticancer effect [21].

5. Summary

Currently, for people with lung cancer, especially those carrying EGFR gene mutations, EGFR-TKIs have emerged as their significant treatment choice. It markedly enhances patient survival and prognosis. However, during treatment, resistance is inevitably developed, including primary resistance and acquired resistance. Acquired resistance can be further classified into two distinct categories: EGFR-dependent and EGFR-independent mechanisms, which hinder the efficacy of drugs. Hence, emerging technologies against EGFR mutations are being actively developed, including fourth-generation EGFR-TKIs (EA1045, LS-106, etc.) that bypass the impact of the EGFR C797S mutation by binding to other sites. There have also been reports of significant efficacy in the combination strategy of osimertinib with other drugs for targeting EGFR mutations. For instance, the combination of the anti-angiogenic drug anlotinib with osimertinib has shown promise in improving the prognosis of NSCLC patients with secondary EGFR mutations. In addition, combining targeted therapy with immunotherapy may yield substantial breakthroughs in the treatment of EGFR mutation-positive tumors. In summary, while some resistance mechanisms associated with EGFR mutations have been identified, many resistance mechanisms are still unknown. Therefore, a thorough comprehension of the molecular foundations of cancer, along with the pursuit of more potent approaches to crafting targeted therapeutic interventions, necessitates ongoing research into potential new drug combinations. This is crucial for overcoming resistance mechanisms and improving patient survival.

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