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Review article

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Progress toward resistance mechanism to epidermal growth factor receptor tyrosine kinase inhibitor

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Abstract: The EGFR signaling pathway plays an important role in the occurrence and development of many malignant tumors. It has become a hot spot in the treatment of advanced cancer. At present, the small molecule epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), has been shown to advanced non-small-cell lung cancer (NSCLC), has a marked drug resistance or has developed one. The EGFR signaling pathway regulates a variety of cellular functions, and its drug resistance may be related to a number of signal transduction pathways, including drug resistance mutations, structural activation, downstream signaling pathway activation and VEGF expression changes, and so on. In this paper, we review the production mechanism of EGFR-TKI drug resistance.

Keywords: non-small-cell lung cancer (NSCLC); epidermal growth factor receptor (EGFR); tyrosine kinase inhibitor

1 Introduction

Lung cancer is responsible for the highest mortality rates of any malignant tumor. Non-small cell lung cancer accounts for 80% - 85% of all lung cancer cases. Chemotherapy for patients with advanced NSCLC is limited with an efficiency of 35% to 20%, and a median survival period of 10 to 12 months [1]. Epidermal growth factor receptor (EGFR) tyrosine kinase is an important target for tumor therapy. Lung cancer patients with the EGFR mutation have high sensitivity to the epidermal growth factor receptor tyrosine kinase inhibitors gefitinib and erlotinib. Relevant research shows that their efficiency rates up to 70% to 80%, and the median survival period is 20 to 30 months [2]. However, resistance to the drug has been found. In clinical practice, the patients' sensitivity to the EGFR-TKI treatments is highly variable, but after about 10 months to 14 months of progression free survival (PFS), they eventually develop resistance to the drug. This is a restriction for the clinical application of the drugs [3]. The drug resistance may be related to a number of signal transduction pathways, including drug resistance mutations, structural activation, downstream signaling pathway activation and VEGF expression changes, and so on. In this review, we focus on the mechanism of EGFR-TKI drug resistance to provide a theoretical basis for clinical treatment.

2 The epidermal growth factor receptor's tyrosine kinase inhibitor and its anti-tumor effect

The epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor on the surface of cell membrane. There are two signal transduction pathways: PI3k-AKT pathway and RAS/RAF-MEK/ERK pathway. It promotes tumor cell proliferation, invasion and angiogenesis. High expression or abnormal activity of EGFR on the cell membrane is an important factor during the formation of many human epithelial tumors, including head and neck squamous cell carcinoma, non-small cell lung cancer, colorectal cancer, breast cancer, and so on. By inhibiting the activity of EGFR, those tumors can be treated. Tyrosine kinase inhibitors (TKIs), belong to

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small molecule quinazoline derivatives, which compete with ATP-binding extracellular ligand binding sites, which block molecular tyrosine autophosphorylation and inhibiting the activation of EGFR, thereby inhibiting cell cycle progression, accelerating cell apoptosis, inhibit angiogenesis, and inhibiting tumor invasion and metastasis. At present, there are two kinds of EGFR-TKI used in clinical applications, gefitinib and erlotinib, which specificity combine with EGFR intracellular tyrosine kinase domain and inhibit its activity, thereby inhibiting the growth of tumor. Gefitinib is a third line single therapeutic drug for advanced non-small cell lung cancer [4]. As an invalid standard regimen treatment of advanced NSCLC, erlotinib has achieved good clinical effects [5]. The high sensitivity of gefitinib and erlotinib are obviously related to the mutation of EGFR, therefore it was called an activating mutation. Most of the sensitive mutations occur in the first four exons of the coding tyrosine kinase (18-21 exon). The deletion mutation of exon 19 and the point mutation of exon 21 were commonly included [6].

3 Drug resistance mechanisms of EGFR tyrosine kinase inhibitors

EGFR tyrosine kinase inhibitors regulate tumor cell proliferation, survival, apoptosis, metastasis, invasion, and tumor-induced angiogenesis through multiple intracellular signal transduction pathways. Therefore, in dependent and non-dependent EFGR signal transduction cancer cells, several molecular mechanisms are important for EGFR inhibitor resistance generation in different cells. These molecular mechanisms are mainly as the following:

4 Epidermal growth factor receptor gene mutation

4.1 Insertion mutation of EGFR exon 20

The insertion mutation of exon 20 accounts for about 4% of EGFR mutations, which is common in non-smoking women with adenocarcinoma [7]. Mutations located between amino acids 768~774 after the EGFR tyrosine kinase region C-spiral and the insertion mutation at amino acid 770 are the most common [8]. Insertion mutations between amino acids 768~774 can lead to the EGFR-TKI and EGFR target binding site being blocked, causing EGFR-TKI primary drug resistance [9].

4.2 Second-site mutations

Second-site mutations include the T790M point mutation in exon 20, the L748S and D761Y point mutations in exon 19, and the T853A point mutation in exon 21 [10]. About 60% of the drug resistance is driven by the EGFR-T790M gatekeeper mutation. To counter the T790M-dependent resistance, the third generation covalent EGFR inhibitors with high potency toward T790M containing mutants and selectivity over WT EGFR have been developed [11].

4.3 K-RAS gene mutation

The K-RAS gene plays a key role in the signal pathway of EGFR through activation of Raf kinase which activates MAPK signal transduction to promote cell proliferation and differentiation. Mutation of the K-RAS GTP hydrolase domain codons 12 and 13 could lead to the continued activation of K-RAS. The NSCLC patients with the mutation are not sensitive to TKIs treatment [12]. K-RAS gene mutation in NSCLC patients with TKIs resistance has been clearly verified [13].

4.4 HER2 gene mutation

Human epidermal growth factor receptor-2 is a member of the epidermal growth factor receptor family. HER2 and EGFR are highly homologous with tyrosine kinase activity. HER2 and EGFR can form a heterodimer to activate tyrosine kinase, which triggers receptor auto-phosphorylation, and activates downstream signal molecules, which promote tumor cell growth, proliferation and differentiation. The study showed that HER2 gene mutations were mainly located in exon 20, mostly in the insertion mutation. The mutation rate was 0.5%, and the mutations were more common in non-smoking women, without adenocarcinoma [14]. Mutant HER2 has stronger receptor activity and signal transduction ability, which can reduce the effect of EGFR-TKI treatment, and lead to EGFR-TKI primary drug resistance [15].

4.5 EML4-ALK fusion gene

Anaplastic lymphoma kinase (ALK) is a member of the insulin receptor tyrosine kinase superfamily, which leads to the activation of the tyrosine kinase domain and promotes the malignant transformation of cells. There were 3% ~ 5% EML4-ALK fusion genes in NSCLC

patients, which were found in young patients with K-RAS, EGFR, BRAF wild type and non-smoking patients with adenocarcinoma [16-18]. EML4-ALK positive patients do not benefit from the treatment of EGFR-TKIs, which may be another mechanism of EGFR-TKIs primary drug resistance.

4.6 B-Raf gene mutation

Vraf murine sarcoma viral oncogene homolog B1 (B-Raf) is the substrate of K-RAS in the EGFR signal pathway. B-Raf encodes a serine / threonine protein kinase in the MAPK pathway, which is involved in the regulation of cell growth, proliferation, and differentiation. B-Raf gene mutations are common in colorectal cancer, melanoma, thyroid cancer, liver cancer, lung cancer, pancreatic cancer and other malignant tumors [19-21]. The B-Raf mutation can lead to 10%~15% K-RAS wild-type non-small cell lung cancer patients developing EGFR-TKI primary drug resistance [22].

4.7 Sustained activation of EGFR downstream signal transduction pathways

PTEN/MMAC/TEP (PTEN) phosphatase function deletion and Akt pathway sustained activation also play a certain role in the process of EGFR inhibitors resistance. PTEN is a kind of tumor suppressor protein which regulates the PI3K/Akt signaling pathway, and the loss of PTEN function leads to excessive activation of the Akt pathway, which increases the anti-apoptotic effect of the cells. Ueda et.al. used three liver carcinoma cell lines (HCC3, CBO12C3 and AD3) and found that gefitinib inhibited Akt phosphorylation in the three cell lines, but in AD3 cell line, the inhibitory effect is lower than HCC3 and CB012C3 cell lines; that PTEN interference RNA (siRNA, siRNA) transfected HCC3 cell line can reduce the sensitivity of gefitinib [23]. Wang et.al. reports that 40% ~ 50% of malignant glioma cells with PTEN deletion, mammalian target of rapamycin (mTOR) inhibitor rapamycin can increase the sensitivity of the EGFR kinase inhibitor [24]. The sustained activation of the PI3K/Akt pathway plays an important role in the development of drug resistance to EGFR inhibitors. Ihle et al. found that the PI3-K signaling pathway inhibitor PX-866 can increase the reactivity of NSCLC patients to EGFR inhibitors, such as gefitinib [25].

4.8 Insulin like growth factor 1 receptor

Insulin-like growth factor 1 receptor (IGF-1R) is expressed widely in tumor cells. IGF-1R plays a key role in differentiation, apoptosis and metastasis of cancer cells [26]. There was a significant correlation between IGF-1R and EGFR inhibitor AG1478 resistance in tumor cells. Through the analysis of EGFR protein expression levels, two primary glioblastomas multiform glioblastoma cell lines are distinct in their sensitivity to AG1478. It was also found that IGF-1R upregulation caused sustained activation of the PI3K-Akt signal and ribosomal protein S6 kinase [27]. In addition, insulin-like growth factor binding protein (IGF-BP) further aggravated gefitinib secondary resistance [28]. Morgillo et al. found that the inhibition of activation of the EGFR downstream pathway mediated by IGF-1R, and can prevent or delay the NSCLC patients from developing gefitinib resistance [29].

5 Other possible mechanisms of drug resistance

5.1 The high expression of HGF (hepatocyte growth factor)

Hepatocyte growth factor (HGF) is a ligand of MET(met proto-oncogene). HGF is able to activate downstream signal of MET, MAPK-ERK1/2 and PI3K-Akt pathways. A previous study showed that HGF can induce acquired resistance to EGFR-TKI [30]. Yano et.al. found that over-expression of HGF and resistance to the primary drug EGFR-TKI is closely related. HGF expression rate was 29% in 44 cases of EGFR-TKI NCSLC patients showing primary drug resistance. The increased expression of HGF through the “bypass pathway”, does not rely on EGFR, but rather than direct activation of the EGFR signaling pathway to reduce the sensitivity of tumor cells to EGFR-TKI [31].

5.2 Laminin-5 overexpression

Laminin(LN) contains one heavy chain-α and two light chains-β, γ. It belongs to the glycoprotein family, and is an important component of basement membrane. Laminin-5 (LN-5) is a member of laminin family and is composed of α3 and β3, γ2 polypeptide chains with two disulfide bonds with the “Y” type glycoprotein. LN-5 plays an important role in the adhesion and migration of growth of tumor

cells, as well as the differentiation of these cells [32]. Katoh et.al. found that LN-5 and EGFR have a common signal pathway. PI3K-AKT and Ras-MAPK are their downstream signal. Increased expression of LN-5 can directly activate the EGFR signal molecule. Inhibitory effects of EGFR-TKI are reduced and induce drug resistance [33].

5.3 MET (met proto-oncogene) amplification

The human c-MET gene is located on the seventh chromosome. Its encoded product is hepatocyte growth factor (HGF) receptor tyrosine kinase. The MET gene amplificies, the mutations and overexpression in many kinds of tumors. MET combined with HGF can activate receptor tyrosine kinase and, promote cell proliferation and differentiation, inducing epithelial cell migration and induced angiogenesis. 20% NSCLC patients have TKIs resistance which is relate to c-MET gene amplification and its occurrence does not correlate with T790M mutations [34,35].

6 Conclusion

With the development of gene detection technology, the study of lung cancer has developed deep into the molecular level, providing new ideas and methods for the individual treatment of lung cancer. At present, EGFR targeted treatment has become a major trend in the treatment of lung cancer, but the resistance mechanism of EGFR-TKIs is still unclear, which has limited its use in clinical treatments. The effect of EGFR-TKI can be improved using a combination of VEGF inhibitors, IGF-1R inhibitors, and other signal pathways inhibitors. The signal transduction of tumor cells is staggered, and a single target drug cannot block all the transduction signals of tumor cells. Therefore, it is a new research trend to develop multi-target therapeutic drugs [36]. Combining other treatments with an antiangiogenic agent may prevent the development of an acquired resistance to EGFR-TKI, and may prolong the duration of the response. Although the mechanism responsible for the additional effect of EGFR-TKI and antiangiogenic agents have not been fully clarified, a recent in vivo study showed that using erlotinib in conjunction with bevacizumab enhanced antitumor activity in T790M mutation-positive or MET-amplified tumors as long as their growth remained significantly suppressed by erlotinib [37]. So far, no single theory can explain the mechanism of EGFR-TKI resistance system. The resistance mechanism of EGFR-TKI and how to overcome resistance is still a topic in the field of cancer research.

Conflict of interest: The authors declare that there is no conflict of interests regarding the publication of this paper.

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