

Research progress on the mechanism and countermeasures of acquired EGFR resistance

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Abstract: Epidermal growth factor receptor (EGFR) -tyrosine kinase inhibitors (TKI) have transformed traditional cancer treatment and brought good news to patients with non-small cell lung cancer. However, drug resistance problems arise one after another. Based on further exploration of the mechanism of EGFR resistance and corresponding therapeutic strategies, the author reviewed relevant literature, classified and summarized it, providing ideas for solving the clinical problem of EGFR resistance and providing hope for tumor patients.

1. Introduction

Lung cancer is a highly lethal malignant tumor in the world, and it is the cancer with the highest incidence and mortality in China [1]. Clinically, it is mainly divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Due to the extremely high degree of malignancy and poor prognosis, NSCLC has become the focus of research on malignant tumors. In recent years, targeted therapy has played an important role in the treatment of advanced NSCLC, showing great benefits. Epidermal growth factor receptor (EGFR) is the first driver gene discovered during the treatment of NSCLC and also the most common driver of NSCLC [2]. Many studies have found that the positive rate of EGFR mutation is very high in patients with advanced NSCLC. Compared with traditional chemotherapy, EGFR-TKI targeted therapy can effectively prolong patients' disease-free progression (PFS) and has the advantage of fewer adverse reactions. The appearance of EGFR-TKI has begun to change the traditional cancer treatment model. [3] Unfortunately, with the extension of treatment time, most NSCLC patients will develop resistance to EGFR-TKI, which not only affects the treatment effect, but also may lead to disease progression (PD). [4] Therefore, it is urgent to solve EGFR resistance. The mechanism and countermeasures of acquired EGFR resistance are reviewed here.

2. Acquired resistance mechanism of EGFR-TKI in non-small cell lung cancer

Acquired resistance to EGFR-TKI appeared. Explanations are as follows: (1) The patient was treated with a single EGFR-TKI inhibitor; (2) Partial response (PR) or complete response (CR) in patients with EGFR-sensitive mutations, or significant and lasting clinical response ≥ 6 months; (3)

systemic disease progression; (4) No other intervention system was performed before EGFR-TKI treatment and new therapy [5]. The first generation of EGFR-TKI acquired drug resistance limited the clinical efficacy. Some scholars [6] found that EGFR T790M mutation at ATP binding site was the main resistance mechanism, and it was not selective to normal tissues and tumor cells. Although the second generation of EGFR-TKI enhanced the inhibition of EGFR mutation, it did not show the effect on EGFR T790M. Therefore, for the resistance mechanism of T790M, people have developed the third generation of Tki-Ocitinib. Ocitinib not only accurately inhibited T790M but retained EGFR wild type (EGFRwt), but also accurately distinguished tissue cells from tumor cells. However, due to the inherent heterogeneity of NSCLC and differences in treatment modalities, acquired resistance to ocitinib eventually occurred

2.1 Targeted gene modified

EGFR-TKIs will develop disease progression after 9-14 months of treatment [7]. The T790M mutation is the most common mechanism of acquired resistance. It was mainly manifested that methionine replaced the 790th threonine of EGFR exon 20 (that is, ACG replaced ATG in position 2369), resulting in a smaller "space" of ATP binding region, which hindered the reversible binding of TKI to EGFR and thus led to drug resistance [7-9]. At the same time, the T790M mutation of EGFR can restore the affinity of the mutant receptor to ATP, thereby reducing the effectiveness of the competing inhibitors. [7]

2.2 C-MYC

Amplification Studies have shown that ocitinib can consistently and rapidly reduce c-Myc levels in the treatment of EGFR-mutated NSCLC. At the same time, c-Myc is inhibited by gene deletion or BET inhibition, and ocitinib resistance can be restored when MYC transcription is inhibited. Therefore, amplification of C-MYC may be the potential mechanism of ocitinib resistance [9]. At the same time, studies have found that ocitinib cannot covalently bind to EGFR due to the presence of C797S mutation, which may lead to drug resistance [10]. More researchers [11] established human lung adenocarcinoma cell line PC-9 in a patient without systematic treatment, set up 15bp deletion of EGFR exon 19, and gradually increased the concentration of ocitinib in the growth medium. Finally, it was found that EGFR ligand overexpression and WT EGFR amplification would cause EGFR hyperphosphorylation. So you have ocitinib resistance. Thus, both C-MYC amplification and mutations in lung adenocarcinoma cell lines PC-9 and C797S may contribute to ocitinib resistance.

2.3 c-MET

Amplification c-MET amplification is another important target of EGFR-TKI resistance. c-MET is an endogenous ligand belonging to the receptor tyrosine protein kinase family, of which Hepato-growth factor (HGF) is the major ligand. MET signaling pathway can cause dysregulation through various mechanisms, including MET amplification or overexpression, such as mutation, rearrangement or amplification, and participate in the process of tumor proliferation and metastasis. C-MET signaling occurs in EGFR-mutated NSCLC through continued activation of MAPK, STAT, and PI3K/ Akt-mediated signaling pathways downstream of EGFR, thus no longer activating EGFR and causing resistance. Meanwhile, the MET ligand HGF has also been shown to induce resistance to EGFR inhibitors. In vitro, HGF activates the PI3K-AKT pathway via GAB1. Studies have found that among the tumor samples from NSCLC patients with EGFR mutations, 61% of the samples were clearly found to have elevated HGF levels after the disease progression of EGFR TKI [12]. It

is suggested that C-MET amplification and HGF increase may be one of the mechanisms leading to EGFR-TKIS resistance.

2.4 Her-2

Amplification HER-2 is also known as erBB-2 gene and belongs to the ErbB receptor family. The mechanism of EGFR-TKI acquired resistance is mainly through the regulation of tumor cells. Studies have shown that [13] HER-2 gene itself has a value-added driving function. In patients with EGFR mutation, amplification of HER-2 can activate signal peptides in the downstream region (through the NEK-ERK and JAK-STST pathways), thereby reducing tumor cells' dependence on EGFR activation of these downstream effector factors. Thus, the antitumor efficacy of EGFR-TKI is decreased. Currently, HER-2 activation is considered to be an important mediator of targeted drug resistance in EGFR-mutated NSCLC.

2.5 Activation of bypass signaling pathway and MAPK pathway

Zhang [14] et al. selected 24 NSCLC patients who underwent targeted therapy to the third generation for biopsy, and found that among these patients, 1 patient had STK11 mutation, 1 patient had PTEN deletion, 1 patient had KRAS mutation, and 2 patients had MET amplification. PIK3CA mutation was present in 3 cases. It has been found that STK11, also known as silk/threonine protein kinase, is a novel tumor suppressor gene and the basic driving factor of resistance to PD-1 axis inhibitors in non-squamous lung adenocarcinoma. PTEN deletion is resistant to first-generation EGFR-TKI, and large PTEN deletion may cause focal progression of oclitinib. Meanwhile, PIK3CA gene contains two mutation regions, E542K and E545K, which can cause acquired drug resistance in NSCLC patients. According to the final data analysis, it was found that the median PFS was significantly shortened in patients with both signal pathway mutations, and there may be a certain synergistic effect between the stronger downstream signals, leading to the occurrence of EGFR-TKIS resistance.

Phosphatidylinositol 3-kinase (PI3K)/Serine/threonine kinase (AKT) and extracellular signal regulatory protein kinases 1 and 2 (ERK 1/2) are also two important signaling pathways. Studies have shown that one of the most frequently activated signal transduction pathways in cancer [15] is the PI3K/ AKT signaling pathway. It not only plays an important role in the occurrence and development of tumor and chemotherapy tolerance, but also has a strong anti-apoptosis ability. To investigate the effect of pct-1 on the resistance of gefitinib to H1299/GR cell lines, we found that, long non-coding RNA (lncRNA)-PCAT-1 can alter the proliferation and apoptotic effects of gefitinib in NSCLC cells by affecting the phosphorylation of AKT, leading to the occurrence of drug resistance [16]. Therefore, we found that activation of many signaling pathways leads to the occurrence of EGFR-TKIS resistance.

3. Treatment strategies for acquired resistance to EGFR-TKIS

The problem of EGFR-targeted therapy resistance in non-small cell lung cancer has brought great trouble to the medical community, and it is urgent to solve the acquired resistance of EGFR-TKIS. The following details are discussed:

3.1 Development of New drugs

Oclitinib is an irreversible EGFR inhibitor that can inhibit T790M resistant mutations, belonging to the third generation of EGFR-Tkis. In combination with platinum-containing chemotherapeutic

agents for T790M mutation-positive NSCLC, the median PFS of osimertinib treatment was significantly extended to 10.1 months (4.1 months for first- and second-generation EGFR-TKIs), and osimertinib was less likely to have grade 3 or higher toxicity. Therefore, in clinical practice, if osimertinib is not selected for first-line treatment, the presence of EGFR mutations should be checked immediately after disease progression and osimertinib should be considered immediately if T790M mutations are present. Currently, osimertinib monotherapy has been established as one of the standard first-line therapies for EGFR-mutation-positive NSCLC [18]. Studies have shown that amivantamab (Evantumab, a humanized bispecific antibody targeting EGFR and C-MET) and lazertinib (Lazertinib, The combination of EGFR-TKI and EGFR-TKI can be used in EGFR-T790m mutation-positive NSCLC treated with EGFR-TKIs. In a Phase I clinical trial, the combined response rate of amivantamab and lazertinib was 36% after treatment with osimertinib in patients with EGFR mutation-positive disease progression [19]. Therefore, we found that the development of new drugs brought new benefits for acquired resistance to EGFR-TKIs.

3.2 Compound Therapy

LS-106 is a novel EGFR inhibitor that inhibits C797S mutation and is evaluated for its antitumor activity in vitro and in vivo. It has been reported that LS-106 is a multi-kinase inhibitor, which can inhibit not only EGFR-C797S mutant kinase, but also RET, ACK1 and other kinases. To better simulate the genetic background of NSCLC, the T790M/C797S mutation of EGFR was tapped into PC-9 cells (EGFR19del) and a tumor cell line PC-9-OR carrying EGFR19del/T790M/C797S was obtained. It was found that LS-106 can strongly inhibit the activation of EGFR19del/T790M/C797S, thus inducing apoptosis of PC-9-OR cells and inhibiting cell proliferation, which proves that LS-106 can inhibit EGFR-TKI resistance caused by C797S [19]. However, the role of this drug in NSCLC resistance still needs more comprehensive research

3.3 C-MET receptor antagonists

In recent years, great gains have been made in the study of MET amplification leading to EGFR resistance in our country. For example, researchers at home and abroad pioneered the idea of MET-TKI+ EGFR-TKI double blocking. A total of 55 subjects were enrolled and treated with a combination of C-MET antagonist (tepotinib) and gefitinib in the experimental group and chemotherapy in the control group. Dual TKI therapy has shown better PFS and OS in MET amplified populations, while reducing the rate of disease progression and mortality. Studies have shown that C-MET receptor antagonists can play a role in EGFR-TKIs resistance caused by C-MET amplification, thus becoming one of the solutions to EGFR resistance [20].

3.4 Combined monoclonal antivasculature therapy

Since EGFR-TKIs and VEGFR-TKIs can act at different stages of tumor growth, the combination of EGFR-TKIs and VegFR-TKIs may offer new hope for patients with advanced lung cancer. Some scholars found that a female patient with egfr exon 19 deletion was treated with Afatinib + bevacizumab instead, and the results showed that SD and OS were 23.5 (after the patient voluntarily abandoned the treatment due to price), with no obvious adverse reactions [21]. This clinical example may not fully demonstrate that TKI combined with monoclonal antibody can overcome EGFR-TKIs resistance, but it is promising. However, many studies have found that combination therapy does not significantly improve patients' PFS [22], so the combination of TKIs with antivasculature drugs in the treatment of EGFR mutated NSCLC remains controversial.

3.5 Studies on combined

Chemotherapy have shown that combined chemotherapy with platinum-containing drugs has a good effect on EGFR-TKIS resistant patients. For example, Jin Qianchen et al. found a female NSCLC patient with repeated drug resistance in Zhongshan Hospital. After eight cycles of treatment with altezumab, bevaccumab, carboplatin and albumin-paclitaxel, Blood gene dynamic monitoring showed that the presence of EGFR mutation decreased significantly, and both liver and lung primary lesions indicated SD, which showed that combined chemotherapy was effective in advanced NSCLC patients with repeated acquired resistance [23].

3.6 Combination of Traditional Chinese Medicine

EGFR-TKIS has a synergistic effect in the treatment of EGFR-mutated NSCLC. The reason is that some scholars have found that the use of Jin Fukang oral liquid, Sijun Guben decoction, Fuzheng Anticancer prescription and other prescriptions can reverse the occurrence of targeted drug resistance, which is synergistic with the treatment of EGFR-TKIS [24].

4. Summary and expectation

The advent of a new generation of sequencing and identification of driver gene EGFR mutations has revolutionized the treatment landscape for patients with NSCLC. Although EGFR-TKIS is the first-line treatment for EGFR-mutation-sensitive NSCLC, with advantages such as fewer side effects and convenient administration routes, the occurrence of acquired drug resistance is extremely easy to cause disease recurrence. At present, there are still many researches on the mechanism of EGFR-TKIS drug resistance, and corresponding treatment strategies are emerging in an endless series. Meanwhile, the emergence of cutting-edge technologies such as whole genome sequencing and RNA sequencing not only enables accurate detection and diagnosis of cancer, but also gives birth to precision medicine and individualized treatment. It is believed that the problem of acquired drug resistance of EGFR-TKI can be solved quickly in the near future, so that EGFR-TKIS can better serve NSCLC patients.

References

- [1] Zhang S, Sun K, Zheng R, et al. Cancer incidence and mortality in China, 2015. *J Nat Cancer Cent*, 2021, 1(1): 2-11.
- [2] Zhang Mengru, Cao Dedong, Ge Wei. Research progress on drug resistance of EGFR-TKIs in non-small cell lung cancer and treatment after drug resistance [J]. *Chinese Journal of Medicine*, 2019, 18(30):50-52+59.
- [3] Yu T, Xia Q, Gong T, et al. Molecular mechanism of acquired drug resistance in the EGFR - TKI resistant cell line HCC827 - TR[J]. 2020.
- [4] Shuaiyong Q U, Chunzheng M A. Mechanism and Research Progress on Anti-tumor Effect of Strengthening the Body Resistance Chinese Herbs[J]. *Chinese Medicine Modern Distance Education of China*, 2018.
- [5] Pan L. Mechanism and therapeutic strategy of EGFR-TKI resistance in non-small cell lung cancer [J]. *Journal of Medical Information*, 2017, 34(07):1-5.
- [6] Park H-R, Kim TM, Lee Y, Kim S, Park S, Ju YS, Kim M, Keam B, Jeon YK, Kim D-W, Heo DS, Acquired resistance to third-generation EGFR tyrosine kinase inhibitors in patients with de novo EGFR T790M-mutant non-small cell lung cancer, *Journal of Thoracic Oncology* (2021).
- [7] Yang L , Ma H , Liu G E . Research progress on the relationship and mechanism of LncRNA in EGFR-TKIs resistance non-small cell lung cancer[J]. *Chinese Journal of Cancer Prevention and Treatment*, 2018, 25(6):449-454.
- [8] Danlei Yu, Wen Zhao, Karin A Vallega, Shiyong Sun, Managing Acquired Resistance to Third-Generation EGFR Tyrosine Kinase Inhibitors Through Co-Targeting MEK/ERK Signaling, *Lung Cancer:Targets and Therapy*(2021).
- [9] Ai Xin, Wang Yan. Research progress of epigenetic mechanism in acquired drug resistance of targeted therapy in non-small cell lung cancer [J]. *Chinese Journal of Lung Cancer*, 2015, 24(10):705-713.

- [10] Pengfei W U, Zhu Y, Yang C, et al. *The Mechanism and Countermeasures on the Secondary Resistance of Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor(EGFR-TKI)[J]. Anti-tumor Pharmacy*, 2015.
- [11] Nishihara S.; Yamaoka T.; Ishikawa F.; Ohmori T, et al; *Third-Generation EGFR-TKI, in Lung Adenocarcinoma Cells with an EGFR-Activating Mutation. Cells*2022, 11, 2201. <https://doi.org/10.3390/cells11142201>
- [12] Carlo, Genova, Erika, et al. *New systemic strategies for overcoming resistance to targeted therapies in non-small cell lung cancer[J]. Expert Opinion on Pharmacotherapy*, 2016.
- [13] Luw W. *EGFR-TKIs in the treatment of non-small cell lung cancer [J]. Journal of Clinical Rational Drug Use*, 2016, 14(10):173-175.
- [14] YujunZhang, Liwen Xiong, Fangfang Xie, Xiaoxuan Zheng, et, al, *Next-generation sequencing of tissue and circulating tumor DNA:Resistance mechanisms to EGFR targeted therapy in a cohort of patients with advanced non-small cell lung cancer, Original Research*, 2021, [doi:10.1003/cam4.3948](https://doi.org/10.1003/cam4.3948).
- [15] Yu Han, Liu Likun, Hao Shulan. *Research progress of PI3K/AKT signaling pathway and Traditional Chinese medicine therapy in colorectal cancer [J]. Clinical Research of Traditional Chinese Medicine*, 2017, 13(35):144-148.
- [16] Shaojia Wang, Chao Liu, Qing Lei, et, al, *Relationship between long non-coding RNA PCAT-1 expression and gefitinib resistance in non-small-cell lung cancer cells, Respiratory Research (2021) 22:146*.
- [17] Tsubata, Y.; Tanino, R.; Isobe, T. *Current Therapeutic Strategies and Prospects for EGFR Mutation-Positive Lung Cancer Based on the Mechanisms Underlying Drug Resistance. Cells*2021, 10, 3192.
- [18] Liang C X, Shi Y K, Han X H. *Progress in research of drug resistance of non-small cell lung cancer to targeted therapy and the countermeasures[J]. Chinese Journal of New Drugs*, 2016.
- [19] Liu Y, Lai M, Li S, et al. *LS-106, a novel EGFR inhibitor targeting C797S, exhibits anti-tumor activities both in vitro and in vivo[J]. Cancer Science*, 2021.
- [20] Liu S Y, Zhang J T, Wu Y L. *Review and interpretation of important clinical studies on non-small cell lung cancer in 2020 [J]. Journal of Evidence-based Medicine*, 201, 21(04):240-249.
- [21] Chen S, Xiang T, Lu W, Hong S, Li Y, Lu Y, Zhang Q, Chen Y, Zhou S, Wang G, Zhang Z, Cai Y. *Combination therapy with afatinib and bevacizumab in an EGFR-mutated non-small cell lung cancer patient with acquired ERBB2 amplification: A case report. Medicine* 2021; 100:8(e24380).
- [22] Li Yong, Long Yong, Zhao Chong, et al. *Efficacy of EGFR-TKIs combined with antiangiogenic drugs in treatment of advanced EGFR-mutant non-small cell lung cancer [J]. Chinese Journal of General Practice*, 2022, 25(08):1007-1013.
- [23] Jin Qianchen, Jiang Jianing, Lv Jinyan, et al. *Atzumab and bevacizumab combined with chemotherapy in the treatment of advanced non-small cell lung cancer after EGFR-TKIs resistance: a case report and literature review [J]. Chinese Journal of Medical Sciences*, 2015, 11(04):239-241.
- [24] Zhang Guixing, Tian Fei, Qu Ziyi, et al. *Research Progress of Traditional Chinese medicine combined with EGFR-TKIS in treatment of non-small cell lung cancer [J]. Henan Journal of Traditional Chinese Medicine*, 2019, 41(08):1281-1284.