Tumor immune microenvironment in non-small cell lung cancer with EGFR mutation before and after EGFR-TKIs treatment

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Abstract

Lung cancer is the most common cancer and a leading cause of death from cancer in men and women in the world. Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) are considered as the first-line treatment of EGFR mutated NSCLC. However, almost all patients eventually develop acquired resistance to EGFR-TKIs, with a median PFS of 9-14 months. With the development of immunotherapy, people realize that the interaction between tumor immune microenvironment (TIME) and tumor cells can also affect EGFR-TKIs treatment. TIME contains a variety of elements and previous researches of TIME in EGFR-TKIs therapy on NSCLC are decentralized. Here, we review the characteristics of TIME in NSCLC from EGFR-TKIs therapy and its role in TKIs resistance.

Tumor immune microenvironment in non-small cell lung cancer with EGFR mutation before and after EGFR-TKIs treatment

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Abstract

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first-line treatment of EGFR mutated NSCLC. However, almost all patients eventually develop acquired resistance to EGFR-TKIs, with a median PFS of 9-14 months. With the development of immunotherapy, people realize that the interaction between tumor immune microenvironment (TIME) and tumor cells can also affect EGFR-TKIs treatment. TIME contains a variety of elements and previous researches of TIME in EGFR-TKIs therapy on NSCLC are decentralized. Here, we review the characteristics of TIME in NSCLC from EGFR-TKIs therapy and its role in TKIs resistance.

Key words

EGFR mutation, NSCLC, EGFR-TKIs, tumor immune microenvironment

Introduction

Lung cancer is the most common cancer and a leading cause of death from cancer in men and women in the world with a 5-year survival rate about 19%[1]. According to the global cancer statistics, the incidence and mortality of lung cancer in the male population ranked first place in the world's malignant tumor, and incidence in the female population ranked third place, mortality ranked second place[2]. In China, the incidence and mortality of lung cancer are the highest in the male population, and the incidence in the female is second place, and mortality is the first place[3].

Epidermal growth factor receptor (EGFR), also known as ERBB1 and HER1, is a transmembrane tyrosine kinase receptor (RTK), which is a member of the human epidermal receptor (HER) family and a crucial component of cell signal pathways[4]. Overexpression and mutation of EGFR can lead to activation of important signaling pathways, such as Ras-MAPK, PI3K-Akt, and STAT, increasing cell proliferation, angiogenesis, and metastasis[5]. About 40% to 80% of patients with non-small cell lung cancer (NSCLC) have EGFR mutation or overexpression[6]. Among them, EGFR mutations were found in 15% of Caucasians and 30-50% of Asian NSCLC patients[7-9]. Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), which are classic small molecule inhibitors can inhibit the EGFR tyrosine kinase domain reversibly through competitive binding with ATP[10]. At present, EGFR-TKIs commonly used in the clinic include gefitinib and erlotinib of the first generation, afatinib and dacomitinib of the second generation, and osimertinib of the third generation. Several clinical trials have shown that EGFR-TKIs can prolong the survival time of patients with tumors harboring EGFR-activating mutations[11-33]. The main clinical trials are shown in Table.

However, patients initially responding to EGFR-TKIs invariably develop resistance and the median progression free survival was about 10 months[11, 16, 23, 30]. Resistance to EGFR-TKIs is inevitable due to various mechanisms, such as the secondary mutation known as T790M(50%)[34]; MET amplification(5%), HER-2 amplification(8%), PI3K mutation(5%), histologic transformation(14%)[35, 36]; new mutations like EGFR-C797S mutation, EGFR-L792H mutation and EGFR-G796R[37-39]. Previous studies focused too much on drug resistance caused by tumor cells, and few on tumor immune microenvironment. Therefore, it is imperative to study the mechanism of tumor immune microenvironment in tumor drug resistance. The tumor immune microenvironment (TIME) contains numerous cell types in addition to cancer cells, which include bone marrow-derived inflammatory cells, lymphocytes, blood vessels, fibroblastic cells, and the extracellular matrix composed of collagen and proteoglycans[40, 41]. Next, this review will describe the TIME of NSCLC with EGFR mutation in five aspects: tumor extracellular matrix; Cancer-associated fibroblasts; Tumor vasculature; tumor infiltrating immune cells; Cytokines secreted by tumor and immune cells.

Extracellular matrix

The extracellular matrix (ECM) is the non-cellular component present within all tissues and organs, and provides not only essential physical scaffolding for the cellular constituents but also initiates crucial biochemical and biomechanical cues that are required for tissue morphogenesis, differentiation, and homeostasis[42]. The interaction between NSCLC tumor cells with EGFR mutation and the ECM is crucial for tumor development (Figure 1A). EGFR mutation can increase twist, snail, and other gene expression in the process of tumor development, which induced epithelial mesenchymal transition (EMT) and promoted tumor cell

invasion and metastasis, EGFR-TKIs can inhibit EMT and prevent tumor cell invasion and metastasis[43, 44]. The activation of EGFR in tumor cells can also increase the activity of matrix metalloproteinase-9 (MMP-9), which can promote the destruction of the ECM barrier and the invasiveness of tumor cells[45, 46]. In recent years, many studies have found that ECM is related to EGFR-TKIs resistance. The ECM of tumor cells with EGFR-TKIs resistance highly expresses integrin- β 1, which is a collagen receptor, and produces a large amount of collagen around the tumor cells. The sensitive cell lines showed EGFR-TKIs resistance after co-cultured with Integrin β -1 which was extracted from drug-resistant cell lines, and inhibition of integrin β -1 expression can reverse EGFR-TKIs resistance[47].

Cancer-associated fibroblasts

Cancer-associated fibroblasts are one of the important components of the tumor microenvironment, which are activated by growth factors, direct intercellular communication, adhesion molecules, and other factors. Different from normal fibroblasts, CAFs are perpetually activated, do not return to normal phenotype and apoptosis, also promote tumor progression[48]. CAFs play an important role in the malignant progression of cancer by releasing growth factors and cytokines which stimulate angiogenesis, cell proliferation, invasion, and metastasis[48]. The function of CAFs in EGFR-TKIs sensitive lung cancer is different from that in EGFR-TKIs resistant lung cancer (Figure 1B). In vitro and in vivo experiments showed that CAFs in EGFR-TKIs resistant lung cancer increased the expression and phosphorylation of ANXA2 by secretion of growth factors HGF and IGF-1 and by activation of the corresponding receptors c-met and IGF-1R, which induced EMT of tumor cells and promoted EGFR-TKIs resistance[49]. In addition, CD200-positive CAFs in tumor tissues can augment the sensitivity of EGFR mutant tumor cells to EGFR TKI[50]. Another study found that podoplanin-positive CAFs promoted tumor cells to EGFR-TKIs resistance and inhibition of the expression of podoplanin in CAFs can reverse the drug resistance[51]. A recent study found that mesenchymal cells isolated from EGFR mutant lung cancer can differentiate into CAFs which can promote tumor invasion and metastasis[52].

Tumor vasculature

Tumor vasculature is another important component of the tumor microenvironment. Tumor vasculature arises from two different biological processes: angiogenesis consisting of the formation of new blood vessels from pre-existing vessels and vasculogenesis consisting of the formation of new blood vessels by recruitment of circulating endothelial progenitor cells[53]. Hypoxia inducible factors (HIFs) are one of the main signals regulating the process of angiogenesis which induce transcription of genes responsible for activation of angiogenesis[54]. Another important regulator is the vascular endothelial growth factor (VEGF) and its receptor (VEGFR), which can stimulate angiogenesis[55]. Tumor vasculature in EGFR mutant lung cancer is different from that in EGFR wild type lung cancer (Figure 1C). It was found that the vascular-poor area in lung adenocarcinoma with EGFR mutation is less than the tumor without EGFR mutation[56]. Another study found that tumors with mutations in exon 20 and 21 of EGFR exhibited a high level expression of VEGFR, while those with mutations in exon 19 of EGFR exhibited a low level expression of VEGFR[57]. For EGFR-TKIs resistant non-small cell lung cancer, heat shock protein 90 (Hsp90) inhibitors can overcome HGF-triggered resistance to EGFR-TKIs by reducing EGFR protein expression and tumor angiogenesis[58]. Another study found that Met activation by HGF stimulated the production of vascular endothelial growth factor (VEGF) and facilitated angiogenesis, which indicated that HGF induced EGFR-TKIs resistance and angiogenesis. Triple inhibition of EGFR, Met, and angiogenesis was useful for controlling the progression of EGFR-mutant lung cancer with HGF-triggered EGFR-TKIs resistance[59]. An in vivo and in vitro experiment on EGFR-TKIs combined with chemotherapy in the treatment of EGFR mutant lung cancer found that EGFR-TKIs combined with chemotherapy can inhibit tumor progression and angiogenesis by down regulating c-MYC and HIF- α pathways[60].

Tumor infiltrating immune cells

At present, the majority of research on tumor immune microenvironment is tumor infiltrating immune cells, and almost All immune cell types can be found in the tumor microenvironment, including tumor associated

macrophages (TAM), dendritic cells (DC), natural killer cells (NK), B lymphocytes (B cell), T lymphocytes (T cell), marrow-derived suppressor cells (MDSC), neutrophils, etc.[61]. The immune cells play an important role in shaping the TIME of EGFR mutant lung cancer by secreting cytokines and activating signal pathways (Figure 2).

Tumor associated macrophages

Macrophage originates from progenitors in the bone marrow and enters the peripheral blood. During homeostasis and inflammation, they migrate into tissues and differentiate into macrophage following exposure to local growth factors, proinflammatory cytokines, and microbial products. The function of macrophages in the tumor is multiple[62]. As for tumor associated macrophages (TAM), studies have found that the number of CD68 positive and CD204 positive TAM in EGFR mutant NSCLC tumor tissues is less than that of EGFR wild type, and the prognosis is better[63]. Moreover, EGFR mutated lung cancer with high infiltrating CD204 positive TAM had high aggressiveness and poor prognosis[64]. Another study found that EGFR-TKIs resistant lung cancer was correlated to tumor infiltrating CD68 positive TAM and S100A9 positive MDSC, which resulted in resistance through the NF- κ B pathway[65]. Two major macrophage subpopulations with different functions include classically activated or inflammatory (M1) and alternatively activated or anti-inflammatory (M2) macrophages have been recognized. M1 macrophage has robust anti-tumoral activity whereas M2 macrophage promotes tumor formation and progression[66]. CD68 positive and CD204 positive TAM is M2 type macrophage. It was found that the level of M2 type macrophage in EGFR-TKIs resistant lung cancer was higher than that in EGFR-TKI sensitive lung cancer[67]. Therefore, it can be inferred that EGFR-TKIs resistance is related to M2 type TAM, and reducing M2 type TAM may reverse EGFR-TKIs resistance.

Dendritic cells

Dendritic cells (DC) are unique immune cells linking innate and adaptive immune responses. They are considered as the most effective antigen presenting cells, which play an important role in the process of tumor antigen recognition and presentation[68]. It was found that the level of major histocompatibility complex class II (MHC II) positive DC in EGFR-TKIs sensitive tumor was higher than that in EGFR-TKIs resistant tumor[69]. Another study found that lung cancer with EGFR mutation inhibited the maturation of DC and induced the production of anergic DC to repress antitumor immunity through exosomes[70]. Analysis of the NSCLC transcriptomic dataset revealed selective overexpression of CD47 in patients with EGFR mutation. EGFR-TKIs significantly reduced CD47 expression on the surface of pre-apoptotic cells, favoring more efficient engulfment of cancer cells by DC. CD47 expression became up-regulated following EGFR-TKIs resistance development, and blocking of this protein by a specific monoclonal antibody increased the clearance of EGFR-TKI resistant cells by phagocytes[71]. Therefore, EGFR-TKIs resistance is associated with decreasing of DC and malfunction of DC.

Natural killer cells

Natural killer cells (NK) are lymphocytes of the innate immune system that monitor cell surfaces of autologous cells for an aberrant expression of major histocompatibility complex class I (MHC I) molecules and cell stress markers, which play an important role in antitumor immune responses[72]. The study found that patients with high expression of NK cells had a greater OS and PFS and NK cells increased after EGFR-TKIs treatment[73]. For EGFR-TKIs resistant lung cancer, up regulating ICAM1 can induce drug resistance, and NK92-CD16 cells combined with the EGFR antibody could reverse the drug resistance[74]. Another study found that gefitinib greatly enhanced NK cells cytotoxicity to lung cancer cells with EGFR L858R+T790M resistance mutation. Gefitinib could block the immune escape by up-regulating the expression of NKG2D ligands ULBP1, ULBP2, or MICA on tumor cells and NKG2D on NK cells in the co-culture system[75]. A combination of EGFR-TKIs and NK cells adoptive immunotherapy may represent a potentially effective strategy for EGFR-TKIs resistant lung cancer.

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSC) are derived from bone marrow and can support tumor progression by promoting tumor cell survival, angiogenesis, invasion of healthy tissue by tumor cells, and metastases[76]. MDSC consist of two large groups of cells termed granulocytic or polymorphonuclear (PMN-MDSC), which are phenotypically and morphologically similar to neutrophils; and monocytic (M-MDSC)—phenotypically and morphologically similar to monocytes[77]. It was found that a high percentage of MDSC in EGFR mutant lung cancer was correlated with poor prognosis and reducing MDSC can improve the prognosis[78]. In addition, S100A9 positive MDSC counts in EGFR-TKIs resistant lung cancer was higher than healthy donors. Further study found that this kind of MDSC could be transformed into M2 type TAM by activating the RELB gene, thus promoting the proliferation and metastasis of tumors and resulting in EGFR-TKIs resistance[65]. At present, there are few studies on the mechanism of MDSC that participated in EGFR-TKIs resistance. Whether MDSC inhibition can reverse EGFR-TKIs resistance needs further study.

Neutrophils

Neutrophils which are the largest number of immune cells in humans have always been considered as uncomplicated front-line troopers of the innate immune system. Recently, studies have found that neutrophils are associated with anti-tumor immune response[79]. Several studies have found that a low neutrophil-to-lymphocyte ratio was a good prognostic factor in EGFR mutant NSCLC patients receiving EGFR-TKIs treatment[80-82]. Another study found that intratumoral neutrophil density was lower in EGFR mutant NSCLC comparing with non-EGFR mutant NSCLC[83]. At present, there are few studies on the relationship between neutrophils and EGFR-TKIs resistance mechanism. Whether neutrophils are related to EGFR-TKIs resistance needs further study.

B lymphocytes

B lymphocytes (B cells) are an important part of the human adaptive immune response and humoral immune response. Studies have found that B cells play an important role in anti-tumor immune response[84]. According to the analysis of the TCGA database, tumor infiltrating B cells (TIB) abundance is significantly higher in the NSCLC with EGFR mutation[85]. Studies have found that B cells usually existed in a tissue called tertiary lymphoid structure (TLS), and TLS was associated with a good prognosis[86]. The main function of B cells is humoral immune response, in lung tissue, B cells differentiate into plasma cells, which produce specific antibodies and recognize tumor associated antigens[86, 87]. In addition, activated TIB can activate tumor infiltrating T cells (TIT) through presenting tumor antigen to TIT and activating the anti-tumor effect of TIT[88]. However, regulatory B cell (Breg), a subtype of B cells, can inhibit immunity and promote tumor proliferation and metastasis[89]. It has been found that Breg can weaken the response of T cells and NK cells and facilitate the immune suppressive activity of regulatory T cells (Treg) by up-regulating immune regulatory ligands through secreting immunosuppressive factors such as IL-10, TGF- β [90]. Τηρεφορε, της ρελαιονσηπ βετωεν Β εελλς ανδ ΕΓΦΡ-TKΙς ις ζομπλςατεδ ανδ νεεδς φυρτηερ στυδψ.

T λψμποςψτες

T λψμποςψτες (T εελλς) αρε αν ιμφορταντ παρτ οφ της ηυμαν αδαπτιε ιμμυνε ρεσπονσε ανδ εελλυλαρ ιμμυνε ρεσπονσε. T εελλς πλψ αν ιμφορταντ ρολε ιν αντι-τυμορ ιμμυνε ρεσπονσε ανδ αρε της μοστ ρεσεαρςηεδ ιμμυνε εελλς ιν TIME[91]. T εελλς ζονσιστ οφ διφφερεντ συβποπυλατιονς, ινζλυδινγ νάιβε T cells that can respond to new antigens, memory T cells that are activated from previous antigens and maintain long-term immunity, and regulatory T cells that regulate immune response[91]. It was found that the percentage of TIT in EGFR mutant NSCLC was lower than that in NSCLC without EGFR mutation, and a low percentage of TIT predicted poor prognosis[92]. Another study found that the number of CD8+CD39+T cells in EGFR mutant NSCLC patients was less than that in EGFR wild-type NSCLC patients. The study suggested that CD39+T cells were related to the good effect of the immune checkpoint inhibitor (ICI), which partly explained the poor effect of EGFR mutated NSCLC on ICI[93]. Moreover, it was found that the number of CD8+ tumor infiltrating T cells in EGFR mutant NSCLC was less than that in EGFR wild type NSCLC[94], and the proportion of PD-L1 + or PD-1 +, CD8 + T cells in CD8 + T cells were also less comparing with non-EGFR mutant NSCLC[95, 96]. This was also confirmed by an animal experiment on genetically engineered mice

with EGFR mutation. The study found that the number of CD8+ TIT in mice with EGFR mutation was less than that with EGFR wild type and other driving gene mutations[97]. T cell immunoglobulin and mucin domain containing protein 3 (TIM3) is a member of the TIM family. Studies have found that the number of TIM3+T cells in the EGFR mutant NSCLC tumor is less than that in EGFR wild type tumor, and T cells with TIM3 positive consisted of CD8+T cells, CD4+T cells, and NKT cells[98].

On the comparison of before and after EGFR-TKIs treatment, the study found that the total T cells, CD4+T cells, and CD8+T cells of patients with EGFR mutation had no significant changes before and after EGFR-TKI treatment, but the ratio of CD4+/CD8+T cells increased, and the high ratio of CD4+/CD8+T cells was associated with poor prognosis[73]. Studies have found that T cell apoptosis was caused by EGFR mutation which increased the expression of PD-L1 through the p-ERK1/2/p-c-jun pathway. EGFR-TKIs prevented T cell apoptosis and inhibit tumor proliferation by decreasing the expression of PD-L1 through inhibiting the EGFR pathway[99]. Another study found that the number of Treg in EGFR mutant NSCLC was less than that in non-EGFR mutant NSCLC, because The EGFR signal activated cJun/cJun N-terminal kinase and reduced interferon regulatory factor-1; the former increased CCL22, which recruits CD4+ regulatory T cells, and the latter decreased CXCL10 and CCL5, which induce CD8+ T cell infiltration. EGFR-TKIs can reverse this phenomenon by inhibiting EGFR signaling pathway[100]. The anti-tumor response of T cells is recognition of the tumor antigen presented by MHC-I. Studies have found that EGFR mutation decreased the expression of MHC-I through the MEK-ERK pathway. EGFR-TKIs increased the expression of MHC-I by inhibiting the EGFR pathway, and recruited T cells to the tumor. Therefore, the number of CD8+T cells in the tumor after EGFR-TKIs treatment is more than that before treatment[101].

Someone used a classification based on the PD-L1 and CD8-positive tumor infiltrating lymphocytes (TIL) statuses. The outcomes of patients with a negative PD-L1 expression and a high CD8-positive TIL count were significantly better than those with other classifications. In patients with positive PD-L1 and high CD8-positive TIL, the rate of EGFR mutation was significantly lower than that in non-EGFR mutation[102]. Moreover, for positive PD-L1 and high CD8+TIL NSCLC with EGFR mutation, the prognosis of EGFR-TKIs is poor, but this type of NSCLC is likely to respond to ICI[103, 104]. A study found that the proportion of PD-L1 positive T cells in peripheral blood of patients with EGFR mutation is less than that in non-EGFR mutation patients. However, the proportion of PD-L1+T cells in peripheral blood of patients with EGFR mutation is significantly increased after the EGFR-TKIs treatment. Moreover, PD-L1+T cells are positively correlated with the poor response of EGFR-TKIs[105]. For uncommon EGFR-mutant tumors, including G719X, L861Q, S768I, and Ex20 ins, the PD-L1 expression in tumor cells was higher than that in common EGFR mutant tumors. Moreover, the proportion of CD8+TIL in uncommon EGFR-mutant tumors was more than that in common EGFR mutant tumors. Patients with high expression of PD-L1 and abundant CD8+TIL exhibited a poor response to EGFR-TKIs, but the better response to ICI[106]. There are many studies about T cells with EGFR mutation and EGFR-TKIs resistance, and the mechanisms of EGFR-TKIs resistance are diverse. Therefore, the therapeutic strategy on T cells is one of the research directions for reversing EGFR-TKIs resistance.

Cytokines secreted by tumor and immune cells

Cytokines are small molecular proteins with extensive biological activities synthesized and secreted by immune cells and non-immune cells through stimulation, including interleukin, interferon, tumor necrosis factor superfamily, colony stimulating factor, chemokines, growth factors, etc.[107], which play an important role in tumor inhibition, proliferation and metastasis (Figure 3). Studies have found that in EGFR mutant lung cancer, alveolar macrophages downregulated surface expression of MHC-II and costimulatory molecules; increased production of CXCL1, CXCL2, IL1 receptor antagonist; and increased phagocytosis. EGFR-TKIs can decrease alveolar macrophages in EGFR mutant lung cancer[108]. It has been found that EGFR-TKIs can induce PD-L1 protein degradation in NSCLC cells with EGFR mutation through the GSK3 β pathway and ubiquitin protease pathway, which contributed to T cell activation[109]. In addition, EGFR mutation can up regulate amphiregulin (AREG) and AREG maintained the Treg cell suppressive function via the EGFR/GSK-3 β /Foxp3 axis, furthermore, inhibition of EGFR by EGFR-TKIs restored the activity of

GSK-3 β and attenuated Treg cell function[110].

As for EGFR-TKIs resistance, it was found that Axl kinase was overexpression in EGFR-TKI resistant NSCLC, which was positively correlated with the expression of genes encoding immune checkpoint molecules (CD274, CTLA4), chemokine receptors (CXCR4, CXCR6), or chemokines (CXCL16). These chemokines can induce tumor invasion and metastasis. Inhibition of Axl kinase activity can reduce the expression of these chemokines[111]. In addition, studies have found that overexpression of hepatocyte growth factor (HGF) induces EMT through the HGF-MET pathway, resulting in EGFR-TKIs resistance[112-120]. MiR-1-3p and miR-206 can reverse HGF-induced EGFR-TKIs resistant lung cancer through inhibition of c-Met signaling and EMT[121]. Overexpression of PD-L1 is one of the mechanisms of EGFR-TKIs primary resistance. Studies have found that PD-L1 led to EGFR-TKIs primary resistance by inducing EMT through the TGF- β /Smad3 pathway. Inhibition of the TGF- β /Smad3 pathway can reverse EGFR-TKIs resistance[122-124]. Moreover, the activation of the TGF- β /Smad2 pathway can induce EMT and EGFR-TKIs resistance by increasing the expression of CXCR4[124, 125]. The study also found that TGF- β can induce EMT and EGFR-TKIs resistance by increasing the expression of PCK α [126]. In addition, TGF- β 1 can induce EGFR-TKIs resistance by activating the Akt-ERK pathway, increasing the expression of MIG6, and decreasing the expression of PTEN[127, 128].

It was found that overexpression of IL-6 in EGFR-mutant NSCLC was correlated with EGFR-TKIs resistance[129]. In addition, the activation of the adrenergic β 2 receptor can induce EGFR-TKIs resistance by increasing the expression of IL-6[130]. For the detection of serum cytokines in NSCLC with EGFR mutation, the study found that patients with high levels of IL-6, VEGF, and HGF in serum had a poor response to EGFR-TKIs treatment. Dynamic change of these cytokines in blood during TKIs treatment can predict the efficacy of EGFR-TKIs[131, 132]. Studies have found that RNF25 promoted EGFR-TKIs resistance in EGFR-mutant NSCLC cells by inducing ERK reactivation through the expression of IL6 via the NK- κ B signal pathway, and inhibition of RNF25 or NF- κ B and ERK pathways can reverse EGFR-TKIs resistance[133]. In addition, studies have found that overexpression of miR-762 which was induced by IL6, promoted EGFR-TKIs resistance in NSCLC through posttranscriptional repression of active BCR related protein (ABR)[134]. Not only IL-6 can induce EGFR-TKIs resistance, but also IL-8 can induce EGFR-TKIs resistance by EMT through activation the MAPK signal pathway[135].

Integrin linked kinase (ILK) regulates the interaction between tumor cells and the extracellular environment, activates signal pathway, promotes cell proliferation, migration and EMT. SHP2 is crucial for the activation of the receptor tyrosine kinase signal pathway. Studies have found that ILK and SHP are highly expressed in EGFR-TKIs resistant NSCLC[136]. Other studies have found that secreted phosphoprotein 1 (SPP1) was significantly increased in EGFR-TKIs resistant lung cancer cells, and inhibition of SPP1 increased sensitivity of lung cancer cells to EGFR-TKIs and decrease the ability of invasion[137]. Overexpression of type 1 insulin-like growth factor receptor (IGF1R) is associated with EGFR-TKIs resistance. Studies have found that overexpression of IGF1R can promote EGFR-TKIs resistance by inducing EMT of tumor cells, and inhibition of IGF1R can reverse EMT of tumor cells and sensitivity to EGFR-TKIs[138]. CXCR7, an atypical G protein-coupled receptor, can promote EGFR-TKIs resistance by EMT of tumor cells through activation of MAPK-ERK pathway via β -arrestin[139]. There are many studies on the relationship between cytokines and EGFR mutation and EGFR-TKIs resistance, involving complex and diverse signaling pathways, which can be classified as one point, namely tumor EMT. Therefore, inhibitors targeting multiple pathways of EMT may be one of the research directions for reversing EGFR-TKIs resistance.

Conclusion

In conclusion, the tumor immune microenvironment of EGFR mutated NSCLC is different from that of non-mutated NSCLC, which is one of the reasons why EGFR mutated NSCLC is a poor response to ICI. The tumor immune microenvironment of EGFR mutated NSCLC also changes during the treatment of EGFR-TKIs. The tumor immune microenvironment of EGFR-TKIs resistance can be summarized into three points: 1. Comparing with EGFR-TKIs sensitive tumor, there are more immunosuppressive cells and fewer immune activated cells in EGFR-TKIs resistant tumor and the tumor microenvironment is an immunosup-

pressive state;2. Tumor cells and immunosuppressive cells secrete many immune negative regulatory factors, inhibit the recognition and presentation of tumor antigen and the antitumor effect of immune cells, resulting in immune escape;3.EGFR-TKIs resistant tumor appears EMT. The above three points interact with each other to form a signal regulatory network, which together leads to EGFR-TKIs resistance. Inhibiting one of them can not fundamentally reverse the EGFR-TKIs resistance. Therefore, future research on reversing EGFR-TKIs resistance needs to invent pan targeted drugs that inhibit the key points of the signal regulatory network, change immune cell infiltration, change immune regulatory factors and reverse EMT, and finally reverse EGFR TKIs resistance.

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Disclosure

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf. We all declare the following: we haven't received support from any organisation for the submitted work; we have no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; and we have no other relationships or activities that could appear to have influenced the submitted work.

Table. Clinical trials of EGFR-TKI in EGFR mutated NSCLC

Authors	Numbers of patients	Groups	mOS(month)	P value	mPFS(month)
MokTS [11]	261	Gefitinib			10
		Carboplatin + paclitaxel			7
Maemondo M [12]	230	Gefitinib	30.5	P _i 0.001	10.8
		Carboplatin + paclitaxel	23.6		5.4
Sun JM [14]	33	Gefitinib			15.7
		Pemetrexed			2.9
Mitsudomi T [15]	172	Gefitinib			9.2
		Docetaxel			6.3
Wu YL [16]	217	Erlotinib	26.3	P=0.607	11
		Gemcitabine + cisplatin	25.5		5.5
Zhou C [17, 18]	154	Erlotinib	22.8	P=0.2663	13.1
		Standard chemotherapy	27.2		4.6
Rosell R [21]	174	Erlotinib	19.3	P=0.87	9.7
		Standard chemotherapy	19.5		5.2
Wu YL [23]	364	Afatinib			11
		Cisplatin + gemcitabine			5.6
Sequist LV [24]	345	Afatinib			11.1
		Cisplatin + pemetrexed			6.9
Wu YL P=0.2224	Mok TS [26, 27]	452	Dacomitinib	34.1	P=0.04
		Gefitinib	26.8		9.2
Soria JC P=0.24	Ramalingam SS [28, 29]	556	Osimertinib	38.6	P=0.04
		Gefitinib	31.8		10.2
Mok TS [30]	419	Osimertinib			10.1
		Pemetrexed + Carboplatin / cisplatin			4.4

Abbreviations: mOS, median overall survival; mPFS, median progression free survival; ORR, objective response rate

Figure 1. The ECM, CAFs and Vasculature in TIME of EGFR mutant lung cancer.

A. The ECM in TIME of EGFR mutant lung cancer, EGFR mutant lung cancer cell overexpresses twist and snail, which induces EMT. EGFR mutant lung cancer cell also overexpresses MMP9, which destructs the ECM barrier. EGFR-TKIs resistant lung cancer cell highly expresses integrin- $\beta 1$. B. The CAFs in TIME of EGFR mutant lung cancer. In EGFR-TKIs sensitive lung cancer, the CAFs highly expresses CD200. In EGFR-TKIs resistant lung cancer, the CAFs highly expresses c-met and IGF-1, which induces tumor cell overexpresses ANXA2 and EMT. The CAFs in TIME of EGFR-TKIs resistant lung cancer also expresses podoplanin. C. Vasculature in TIME of EGFR-TKIs resistant lung cancer. The expression of HGF promotes the activation of MET, which stimulates the production of VEGF. The activation of c-MYC and

overexpression of HIF- α also induces EGFR-TKIs resistance.

Figure 2. Immune cells in TIME of EGFR mutant lung cancer.

The ratio of neutrophil, MDSC, CD68+ and CD204+ TAM(M2) in EGFR-TKIs sensitive lung cancer is less than that in EGFR-TKIs resistant lung cancer. However, the number of MHC II+DC and NK in EGFR-TKIs sensitive lung cancer is more than that in EGFR-TKIs resistant lung cancer. S100A9+MDSC transforms into M2 type TAM by activating RELB gene, and TAM(M2) induces EGFR-TKIs resistance through the NF- κ B pathway. The EGFR-TKIs resistant lung cancer cell expresses CD47 which blocks the phagocytosis and antigen presentation of DC. Up regulating the expression of ULBP1,2, MICA on tumor cell and NKG2D on NK can promote NK cytotoxicity to tumor cell. B cell exists in the TLS which is associated with a good prognosis. B cell also differentiates into plasma cell and produces specific antibodies and recognizes tumor associated antigens. Breg can weaken the response of T cells and NK cells and facilitate the immune suppressive activity of regulatory T cells (Treg) by secreting immunosuppressive factors. The number of T cell like CD39+, CD8+ or PD-1+, TIM3+ T cell in EGFR mutant lung cancer is less than that in EGFR wild type lung cancer. T cell apoptosis was caused by EGFR mutation which increased the expression of PD-L1 through the p-ERK1/2/p-c-jun pathway. The number of Treg in EGFR mutant NSCLC was less than that in non-EGFR mutant NSCLC. The EGFR signal activates cJun/cJun N-terminal kinase and reduces interferon regulatory factor-1; the former increases CCL22, which recruits CD4+ regulatory T cells, and the latter decreases CXCL10 and CCL5, which induce CD8+ T cell infiltration. EGFR mutation also decreases the expression of MHC-I through the MEK-ERK pathway.

Figure 3. Cytokines in TIME of EGFR mutant lung cancer.

EGFR-TKIs can induce PD-L1 protein degradation through the GSK3 β pathway and ubiquitin protease pathway, which contributes to T cell activation. EGFR mutation can up regulate amphiregulin (AREG) and AREG maintains the Treg cell suppressive function via the EGFR/GSK-3 β /Foxp3 axis. Axl kinase, genes encoding immune checkpoint molecules (CD274, CTLA4), chemokine receptors (CXCR4, CXCR6), or chemokines (CXCL16) are overexpression in EGFR-TKI resistant NSCLC. Overexpression of hepatocyte growth factor (HGF) induces EMT through the HGF-MET pathway, resulting in EGFR-TKIs resistance. PD-L1 induces EMT through the TGF- β /Smad3 pathway. Activation of the TGF- β /Smad2 pathway can also induce EMT by increasing the expression of CXCR4. Furthermore, TGF- β can induce EMT and EGFR-TKIs resistance by increasing the expression of PCK α , activating the Akt-ERK pathway, increasing the expression of MIG6, and decreasing the expression of PTEN. Adrenergic β 2 receptor can induce EGFR-TKIs resistance by increasing the expression of IL-6. RNF25 promoted EGFR-TKIs resistance by inducing ERK reactivation through the expression of IL6 via the NF- κ B signal pathway. ILK, SHP, SPP1, IGF1R are highly expressed in EGFR-TKIs resistant NSCLC. CXCR7 can promote EGFR-TKIs resistance by EMT of tumor cells through activation of MAPK-ERK pathway via β -arrestin.

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