



Tumor immune microenvironment in epidermal growth factor receptor-mutated non-small cell lung cancer before and after epidermal growth factor receptor tyrosine kinase inhibitor treatment: a narrative review

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Objective: To review and summarize the characteristics of the tumor immune microenvironment (TIME) in EGFR-mutated non-small cell lung cancer (NSCLC) after EGFR-TKI treatment and its role in TKI resistance.

Background: Lung cancer is one of the most commonly diagnosed cancer and the leading cause of death from cancer in both men and women around the world. Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) are considered a first-line treatment for EGFR-mutated NSCLC. However, almost all patients eventually develop acquired resistance to EGFR-TKIs, with a median progression-free survival (PFS) of 9–14 months. As immunotherapy has developed, it has become apparent that interactions between the TIME and tumor cells also affect EGFR-TKI treatment. The TIME comprises a variety of components but previous studies of the TIME following EGFR-TKI therapy of NSCLC are inconsistent. Here, we reviewed the characteristics of the TIME in NSCLC after EGFR-TKI treatment and its role in TKI resistance.

Methods: PubMed, Embase, and Web of Science were searched to July 1, 2021 with the following key words: “NSCLC”, “EGFR”, and “immunotherapy”.

Conclusions: The TIME of EGFR-mutated NSCLC is different from that of non-mutated NSCLC, an explanation for EGFR-mutated NSCLC displaying a poor response to ICIs. The TIME of EGFR-mutated NSCLC also changes during treatment with EGFR-TKIs. The TIME in EGFR-TKI-resistant lung cancer can be summarized as follows: (I) compared with EGFR-TKI-sensitive tumors, EGFR-TKI-resistant tumors have a greater number of immunosuppressive cells and fewer immune-activated cells, while the tumor microenvironment is in an immunosuppressive state; (II) tumor cells and immunosuppressive cells secrete multiple negative immune regulatory factors, inhibit the recognition and presentation of tumor antigens and the antitumor effect of immune cells, resulting in immune escape; 3. EGFR-TKI-resistant tumors promote EMT. These three characteristics interact, resulting in a regulatory signaling network, which together leads to EGFR-TKI resistance.

Keywords: Non-small cell lung cancer (NSCLC); epidermal growth factor receptor mutation (EGFR mutation); epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs); tumor immune microenvironment (TIME)

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Introduction

Lung cancer is one of the most commonly diagnosed cancer and the leading cause of death from cancer in both men and women around the world, with a 5-year survival rate of approximately 21% (1). According to global cancer statistics, the incidence of lung cancer and mortality in the male population ranks first globally for malignant tumors, while its incidence in the female population ranks third, and mortality ranks second (2). In China, the incidence of lung cancer and the mortality rate are highest in the male population, while the incidence in females is second, with the highest mortality rate (3).

Epidermal growth factor receptor (EGFR), also known as ERBB1 and HER1, is a transmembrane tyrosine kinase receptor (RTK) and a member of the human epidermal receptor (HER) family, representing 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, resulting in increased cell proliferation, angiogenesis, and metastasis (5). Approximately 40–80% of patients with non-small cell lung cancer (NSCLC) have an EGFR mutation or it is overexpressed (6). EGFR mutations have been found in 15% of Caucasian and 30–50% of Asian NSCLC patients (7-9). Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), classic small molecule inhibitors, inhibit the EGFR tyrosine kinase domain reversibly through competitive binding with ATP (10). At present, EGFR-TKIs commonly used in the clinic include the first generation treatments gefitinib and erlotinib, second generation afatinib and dacomitinib, and osimertinib, a third-generation treatment. Several clinical trials have demonstrated that EGFR-TKIs can prolong the survival of patients with tumors harboring EGFR-activating mutations (11-33). The principal clinical trials are displayed in *Table 1*.

However, patients initially responding to EGFR-TKIs invariably develop resistance, resulting in median progression-free survival (PFS) of approximately 10 months (11,16,23,30).

Resistance to EGFR-TKIs is inevitable due to a number of mechanisms, including the secondary mutation known as T790M (50%) (34), MET amplification (5%), HER-2 amplification (8%), PI3K mutation (5%), histologic transformation (14%) (35,36), and new mutations such as EGFR-C797S, EGFR-L792H, and EGFR-G796R (37-39). There has been too great a focus in previous studies on drug resistance caused by tumor cells, and too little on the tumor immune microenvironment (TIME). It is therefore imperative that the mechanisms of the TIME are studied regarding tumor drug resistance. The TIME comprises numerous cell types in addition to cancer cells, including bone marrow-derived inflammatory cells, lymphocytes, fibroblastic cells, in addition to extracellular matrix (ECM) composed of collagen and proteoglycans (40,41). The review will describe in detail the TIME of EGFR-mutated NSCLC from five perspectives: tumor-infiltrating immune cells; cytokines secreted by tumor and immune cells; tumor ECM; cancer-associated fibroblasts (CAFs); and the tumor vasculature.

We present the following article in accordance with the Narrative Review checklist (available at <https://dx.doi.org/10.21037/tlcr-21-572>).

Tumor-infiltrating immune cells

The majority of research on the TIME at present has focused on tumor-infiltrating immune cells. Almost all immune cell types can be found in the tumor microenvironment, including tumor-associated macrophages (TAMs), dendritic cells (DCs), natural killer cells (NKs), B lymphocytes (B cells), T lymphocytes (T cells), marrow-derived suppressor cells (MDSCs), neutrophils, etc. (42). Immune cells play an important role in shaping the TIME in EGFR-mutant lung cancer by secreting cytokines and activating signal pathways (*Figure 1*).

TAMs

Macrophages originate from progenitors in the bone

Table 1 Clinical trials of EGFR-TKI in EGFR mutated NSCLC

Authors	Numbers of patients	Groups	mOS (month)	P value	mPFS (month)	P value	ORR	P value
Mok TS (11)	261	Gefitinib			10	<0.001	71.20%	<0.001
		Carboplatin + paclitaxel			7		47.30%	
Maemondo M (12)	230	Gefitinib	30.5	<0.001	10.8	<0.001	73.70%	<0.001
		Carboplatin + paclitaxel	23.6		5.4		30.70%	
Sun JM (14)	33	Gefitinib			15.7	<0.01	87.50%	<0.01
		Pemetrexed			2.9		38.90%	
Mitsudomi T (15)	172	Gefitinib			9.2	<0.0001	62.10%	<0.0001
		Docetaxel			6.3		32.20%	
Wu YL (16)	217	Erlotinib	26.3	0.607	11	<0.0001	62.70%	<0.0001
		Gemcitabine + cisplatin	25.5		5.5		33.60%	
Zhou C (17,18)	154	Erlotinib	22.8	0.2663	13.1	<0.0001	83%	<0.0001
		Standard chemotherapy	27.2		4.6		36%	
Rosell R (21)	174	Erlotinib	19.3	0.87	9.7	<0.0001		
		Standard chemotherapy	19.5		5.2			
Wu YL (23)	364	Afatinib			11	<0.0001	66.90%	<0.0001
		Cisplatin + gemcitabine			5.6		23%	
Sequist LV (24)	345	Afatinib			11.1	P0.001	56%	0.001
		Cisplatin + pemetrexed			6.9		23%	
Wu YL & Mok TS (26,27)	452	Dacomitinib	34.1	0.0438	14.7	<0.0001	75%	0.2224
		Gefitinib	26.8		9.2		70%	
Soria JC & Ramalingam SS (28,29)	556	Osimertinib	38.6	0.046	18.9	<0.001	80%	0.24
		Gefitinib	31.8		10.2		76%	
Mok TS (30)	419	Osimertinib			10.1	<0.001	71%	<0.001
		Pemetrexed + carboplatin/ cisplatin			4.4		31%	

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; NSCLC, non-small cell lung cancer; mOS, median overall survival; mPFS, median progression free survival; ORR, objective response rate.

marrow and enter the peripheral blood. During homeostasis and inflammation, they migrate into tissues and differentiate into macrophages following exposure to local growth factors, proinflammatory cytokines, and microbial products. Macrophages have multiple functions in tumors (43). Studies have found that the number of CD68+ and CD204+ TAMs in EGFR-mutant NSCLC tumor tissues is less than that observed in tumors with wild-type EGFR, with a superior prognosis (44). Furthermore, EGFR-mutated lung cancer with high levels of infiltrating CD204+ TAMs was

found to be highly aggressive with poor prognosis (45). In an additional study, it was observed that the resistance of lung cancer to EGFR-TKIs was correlated with the tumor infiltration of CD68+ TAMs and S100A9+ MDSCs, causing resistance through the NF- κ B pathway (46). Two major macrophage subpopulations with different functions include the classically activated or inflammatory (M1) phenotype and the alternatively activated or anti-inflammatory (M2) type. M1 macrophages exhibit robust anti-tumoral activity whereas M2 macrophages promote tumor formation and

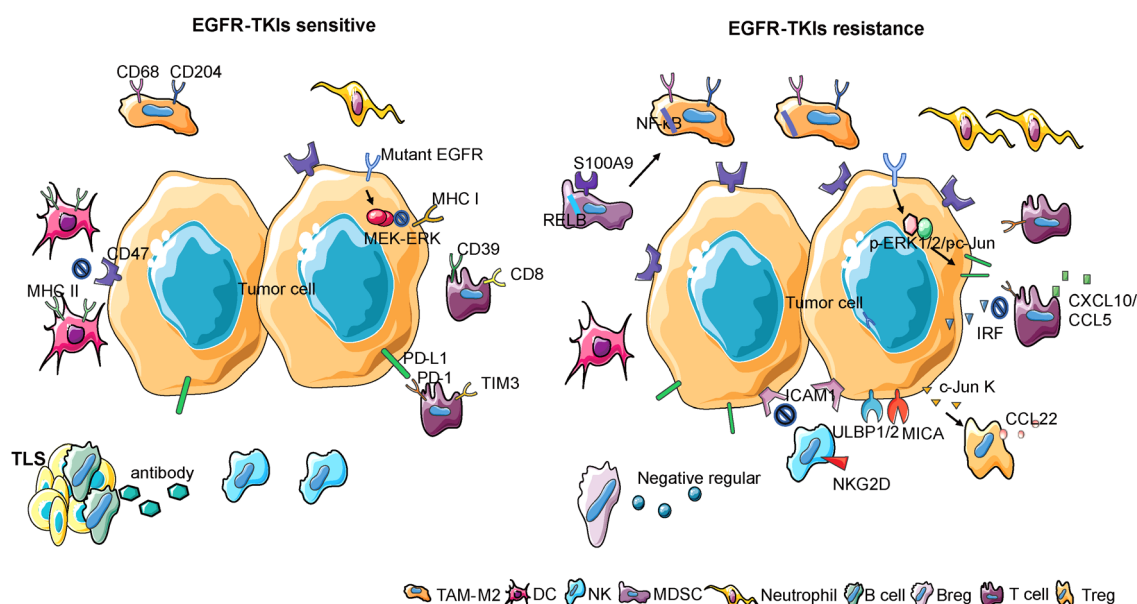


Figure 1 Immune cells in the TIME of EGFR mutant lung cancer. The number of neutrophils, MDSCs, and CD68+ and CD204+ TAMs (M2) in EGFR-TKI-sensitive lung cancer is less than that in EGFR-TKI-resistant lung cancer. However, the number of MHC II+ DC and NK cells in EGFR-TKI-sensitive lung cancer is more than in EGFR-TKI-resistant lung cancer. S100A9+ MDSCs transform into M2 type TAMs by activation of the RELB gene, while TAMs (M2) induce EGFR-TKI resistance via the NF- κ B pathway. EGFR-TKI-resistant lung cancer cells express CD47 which blocks the phagocytosis and antigen presentation by DCs. Up-regulation of ULBP1, 2, and MICA expression on tumor cells and NKG2D on NK cells can promote NK cytotoxicity toward tumor cells. B cells are present within a TLS associated with good prognosis. B cells also differentiate into plasma cells, producing specific antibodies and recognizing tumor-associated antigens. Breg weakens the response of T cells and NK cells, facilitating the immune-suppressive activity of regulatory T cells (Tregs) by secreting immunosuppressive factors. The number of T cell-like CD39+ and CD8+ or PD-1+ and TIM3+ T cells in EGFR-mutant lung cancer are fewer than in wild-type EGFR lung cancer. T cell apoptosis is caused by an EGFR mutation that increases the expression of PD-L1 via the p-ERK1/2/p-c-jun pathway. The number of Tregs in EGFR-mutant NSCLC was fewer than in non-EGFR mutant NSCLC. EGFR signaling activates cJun/cJun N-terminal kinase and reduces interferon regulatory factor-1. The former increases CCL22 expression, which recruits CD4+ regulatory T cells, and the latter decreases CXCL10 and CCL5 expression, which induces CD8+ T cell infiltration. EGFR mutations also decrease the expression of MHC-I via the MEK-ERK pathway. TIME, tumor immune microenvironment; EGFR, epidermal growth factor receptor; MDSC, marrow-derived suppressor cell; TKI, tyrosine kinase inhibitor; MHC, major histocompatibility complex; NK, natural killer; TAM, tumor-associated macrophage; DC, dendritic cell; NSCLC, non-small cell lung cancer.

progression (47). CD68+ and CD204+ TAMs are M2 macrophages. It has been found that the proportion of M2 macrophages in EGFR-TKI resistant lung cancer is higher than in EGFR-TKI sensitive lung cancer (48). Therefore, it can be inferred that EGFR-TKI resistance is related to M2-type TAMs, and that reducing the number of M2 TAMs may reverse EGFR-TKI resistance.

DCs

DCs are unique immune cells that link the innate and adaptive immune response. They are considered the

most effective type of antigen-presenting cell, playing an important role in the process of tumor antigen recognition and presentation (49). It has been found that the proportion of major histocompatibility complex class II (MHC-II)-positive DCs in EGFR-TKI sensitive tumors is higher than in EGFR-TKI resistant tumors (50). Another study found that lung cancer with the EGFR mutation inhibits the maturation of DCs and induces the production of anergic DCs that represses antitumor immunity through exosomes (51). Analysis of NSCLC transcriptomic datasets reveal selective overexpression of CD47 in patients with the EGFR mutation. EGFR-TKIs significantly reduce

CD47 expression on the surface of pre-apoptotic cells, favoring more efficient engulfment of cancer cells by DCs. CD47 expression becomes up-regulated following the development of EGFR-TKI resistance, and blockade of this protein using a specific monoclonal antibody increases the clearance of EGFR-TKIs resistant cells by phagocytes (52). Therefore, EGFR-TKI resistance is associated with decreased numbers of DCs and their dysfunction.

NK cells

NK cells are lymphocytes of the innate immune system that monitor the cell surface of autologous cells for aberrant expression of MHC-I molecules and cell stress markers, which play an important role in the antitumor immune response (53). The study found that patients with a high number of NK cells had greater overall survival (OS) and PFS. The number of NK cells was found to increase after EGFR-TKI treatment (54). For EGFR-TKI resistant lung cancer, up-regulation of ICAM1 was found to induce drug resistance, while NK92-CD16 cells combined with the EGFR antibody were found to reverse drug resistance (55). Another study found that gefitinib greatly enhanced NK cell cytotoxicity toward lung cancer cells with the EGFR L858R+T790M resistance mutation. Gefitinib has been shown to block immune escape by up-regulation of the expression of the NKG2D ligands ULBP1, ULBP2, or MICA on tumor cells and NKG2D on NK cells in a co-culture system (56). A combination of EGFR-TKIs and NK cell adoptive immunotherapy possibly represents an effective strategy for EGFR-TKI resistant lung cancer.

Myeloid-derived suppressor cells (MDSCs)

MDSC arise from the bone marrow and support tumor progression by the promotion of tumor cell survival, angiogenesis, invasion of healthy tissue by tumor cells, and metastases (57). There are two large groups of MDSCs, termed granulocytic or polymorphonuclear MDSCs (PMN-MDSCs), phenotypically and morphologically similar to neutrophils, and monocytic MDSCs (M-MDSC), phenotypically and morphologically similar to monocytes (58). It has been found that the presence of a high number of MDSCs in EGFR-mutant lung cancer was correlated with poor prognosis, and a reduction in MDSC numbers able to improve prognosis (59). In addition, the numbers of S100A9-positive MDSCs in EGFR-TKI-

resistant lung cancer were found to be higher than in healthy donors. Further study has found that this type of MDSC can be transformed into an M2 TAM by activation of the RELB gene, thus promoting the proliferation and metastasis of tumors and resulting in EGFR-TKI resistance (46). Few studies have so far been published on the mechanism by which MDSCs participate in EGFR-TKI resistance. Whether inhibition of MDSCs can reverse EGFR-TKI resistance requires additional study.

Neutrophils

Neutrophils represent the largest cohort of immune cells and in humans are generally considered a non-complex front-line defender of the innate immune system. Recent studies have demonstrated that neutrophils are also associated with the anti-tumor immune response (60). Several studies have shown that a low neutrophil-to-lymphocyte ratio was a good prognostic factor in EGFR-mutant NSCLC patients receiving EGFR-TKI treatment (61-63). Another study demonstrated that intratumoral neutrophil density was lower in EGFR-mutant NSCLC in comparison with non-EGFR mutant NSCLC (64). Few studies have reported on the mechanism and relationship between neutrophils and EGFR-TKI resistance. Whether neutrophils are related to EGFR-TKI resistance requires additional study.

B cells

B cells are an important element of the human adaptive and humoral immune response. Studies have demonstrated that B cells play an important role in the anti-tumor immune response (65). According to analysis using data from the TCGA database, the abundance of tumor-infiltrating B cells (TIBs) is significantly higher in NSCLC with the EGFR mutation (66). Studies have found that B cells are usually found within an ectopic tissue called the tertiary lymphoid structure (TLS), the existence of which is associated with good prognosis (67). The principal function of B cells is participation in the humoral immune response. In lung tissue, B cells differentiate into plasma cells, which produce specific antibodies and recognize tumor-associated antigens (67,68). In addition, activated TIBs can activate tumor-infiltrating T cells (TITs) by being presenting them with tumor antigens and activation of the TIT anti-tumor effect (69). However, regulatory B cells (Bregs), a subtype of B cells, can inhibit immunity and promote tumor

proliferation and metastasis (70). It has been found that Bregs can weaken the response of T cells and NK cells and facilitate the immune-suppressive activity of regulatory T cells (Tregs) by up-regulation of immunoregulatory ligands through secretion of immunosuppressive factors such as IL-10 and TGF- β (71). The relationship between B cells and EGFR-TKIs is complex and requires additional study.

T cells

T cells are an important component of the human adaptive and cellular immune response. They play an important role in the anti-tumor immune response and are the most studied immune cell in the TIME (72). T cells consist of different subpopulations, including naïve 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 the immune response (72). With the breakthrough of immunotherapy in the cancer treatment, oncologists began to explore whether immunotherapy could be applied to EGFR mutant NSCLC. Unfortunately, clinical evidence showed that most of the patients with EGFR-mutated NSCLC would not benefit from the immunotherapy (73-75). It has been found that the percentage of TITs in EGFR-mutant NSCLC was lower than in non-EGFR mutated-NSCLC, while a low percentage of TITs is predictive of poor prognosis (76). Another study found that the number of CD8⁺ CD39⁺ T cells in EGFR mutant-NSCLC patients was less than that in wild-type EGFR NSCLC patients. The study suggested that CD39⁺ T cells were associated with a positive effect of immune checkpoint inhibitors (ICIs) (77). Furthermore, it was found that the number of CD8⁺ TIT cells in EGFR-mutant NSCLC was less than that in wild-type EGFR NSCLC (78), and the proportion of PD-L1⁺ or PD-1⁺ in the CD8⁺ T cell population was also less than non-EGFR mutant NSCLC (79,80). This was also confirmed by animal experiments of genetically engineered mice with the EGFR mutation. The study found that the number of CD8⁺ TITs in mice with the EGFR mutation was less than in mice with wild-type EGFR or other gene mutations (81). These studies partially explained the poor efficacy of EGFR mutant NSCLC when using ICIs. 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 EGFR-mutant NSCLC tumors is fewer than in wild-type EGFR tumors, while T cells that are TIM3 positive can be CD8⁺ T cells, CD4⁺ T

cells, or NKT cells (82).

When comparing patients before and after EGFR-TKI treatment, studies have found that the total number of T cells, and the number of CD4⁺ and CD8⁺T cells in patients with the EGFR mutation were not significantly different, but the ratio of CD4⁺/CD8⁺ T cells increased, a high ratio being associated with poor prognosis (54). Studies have found that T cell apoptosis was caused by the EGFR mutation which increased the expression of PD-L1 through the p-ERK1/2/p-c-jun pathway. EGFR-TKIs prevent T cell apoptosis and inhibit tumor proliferation by decreasing the expression of PD-L1 through inhibition of the EGFR pathway (83). Another study found that the number of Tregs in EGFR-mutant NSCLC was less than in non-EGFR mutant NSCLC, due to EGFR signaling causing activation of cJun/cJun N-terminal kinase and reducing the quantity of interferon regulatory factor-1. The former increased CCL22 expression, which recruits CD4⁺ regulatory T cells, and the latter decreased CXCL10 and CCL5 expression, which induce CD8⁺ T cell infiltration. EGFR-TKIs can reverse this phenomenon by inhibiting the EGFR signaling pathway (84). The anti-tumor response of T cells occurs as a result of the recognition of the tumor antigen presented by MHC-I. Studies have found that the EGFR mutation decreases the expression of MHC-I via the MEK-ERK pathway. EGFR-TKIs increase the expression of MHC-I by inhibiting the EGFR pathway, and recruit T cells to the tumor. Therefore, the number of CD8⁺ T cells in the tumor following EGFR-TKI treatment is greater than prior to treatment (85).

A classification based on PD-L1 and CD8-positive tumor-infiltrating lymphocyte (TIL) status has been proposed. The outcomes of patients with negative PD-L1 expression and a high CD8⁺ TIL count were found to be significantly superior to patients that did not. In patients with positive PD-L1 and high numbers of CD8⁺ TIL, the rate of EGFR mutation was significantly lower than in the non-EGFR mutation (86). Moreover, for positive PD-L1 and high CD8⁺TIL NSCLC with the EGFR mutation, the prognosis after EGFR-TKI therapy is poor, but this type of NSCLC is likely to respond to ICI (87,88). A different study found that the proportion of PD-L1 positive T cells in the peripheral blood of patients with the EGFR mutation was less than in patients without the EGFR mutation. However, the proportion of PD-L1⁺ T cells in the peripheral blood of patients with the EGFR mutation was significantly higher after EGFR-TKIs treatment. Moreover, PD-L1⁺ T cells are positively correlated with the poor

response of EGFR-TKIs (89). For uncommon EGFR-mutant tumors, including G719X, L861Q, S768I, and Ex20 ins, PD-L1 expression in tumor cells was higher than in common EGFR mutant tumors. Moreover, the proportion of CD8⁺ TIL in uncommon EGFR-mutant tumors was greater than in common EGFR mutant tumors. Patients with high PD-L1 expression and abundant CD8⁺TIL exhibit a poor response to EGFR-TKIs, but respond better to ICIs (90). Many studies have been published in which the mechanisms of EGFR-TKI resistance are diverse in patients with the EGFR mutation. Therefore, therapeutic strategies involving T cells represent a promising field of research for reversal of EGFR-TKI resistance.

Cytokines secreted by tumor and immune cells

Cytokines are small molecular proteins with diverse biological properties, and are synthesized and secreted by both immune and non-immune cells following stimulation, including interleukins, interferon, members of the tumor necrosis factor superfamily, colony-stimulating factors, chemokines, growth factors, etc. (91). They play an important role in tumor inhibition, proliferation, and metastasis (Figure 2). Studies have found that in EGFR-mutant lung cancer, alveolar macrophages downregulate surface expression of MHC-II and costimulatory molecules, they exhibit increased production of CXCL1, CXCL2, and IL1 receptor antagonist, and display increased levels of phagocytosis. EGFR-TKIs decrease the number of alveolar macrophages in EGFR-mutant lung cancer (92). It has been found that EGFR-TKIs induce PD-L1 protein degradation in EGFR-mutated NSCLC cells via the GSK3 β and ubiquitin protease pathways, contributing to T cell activation (93). In addition, EGFR mutations can upregulate amphiregulin (AREG) which maintains Treg suppression via the EGFR/GSK-3 β /Foxp3 axis. Furthermore, inhibition of EGFR by EGFR-TKIs restores GSK-3 β activity and attenuates Treg cellular function (94).

It has been found that Axl kinase is overexpressed in EGFR-TKI resistant NSCLC, and is positively correlated with the expression of genes encoding immune checkpoint molecules (CD274, CTLA4), chemokine receptors (CXCR4, CXCR6), and chemokines (CXCL16). Chemokines can induce tumor invasion and metastasis. Inhibition of Axl kinase activity can reduce the expression of these chemokines (95). In addition, studies have found that overexpression of hepatocyte growth factor (HGF) induces EMT through the HGF-MET pathway, resulting

in EGFR-TKI resistance (96-104). MiR-1-3p and miR-206 can reverse HGF-induced EGFR-TKI-resistant lung cancer through inhibition of c-Met signaling and EMT (105). Overexpression of PD-L1 is a mechanism of primary EGFR-TKI resistance. Studies have found that this resistance occurs by induction of EMT via the TGF- β /Smad3 pathway. Inhibition of the TGF- β /Smad3 pathway reverses EGFR-TKI resistance (106-108). Moreover, activation of the TGF- β /Smad2 pathway induces EMT and EGFR-TKI resistance by increasing the expression of CXCR4 (108,109). An additional study found that TGF- β can also induce EMT and EGFR-TKI resistance by increasing the expression of PKC α (110). In addition, TGF- β 1 can induce EGFR-TKI resistance by activating the Akt-ERK pathway, increasing the expression of MIG6, and decreasing the expression of PTEN (111,112).

It has been found that overexpression of IL-6 in EGFR-mutant NSCLC was correlated with EGFR-TKI resistance (113). In addition, activation of the adrenergic β 2 receptor can induce EGFR-TKI resistance by increasing the expression of IL-6 (114). In NSCLC patients with the EGFR mutation, the study found that high levels of IL-6, VEGF, and HGF in serum were associated with a poor response to EGFR-TKI treatment. Dynamic changes in these cytokines in blood during TKI treatment predicted the efficacy of EGFR-TKIs (115,116). Studies have found that RNF25 promotes EGFR-TKI resistance in EGFR-mutant NSCLC cells by inducing ERK reactivation through the expression of IL6 via the NK- κ B signaling pathway, while inhibition of RNF25 or the NF- κ B and ERK pathways can reverse EGFR-TKI resistance (117). In addition, studies have found that overexpression of miR-762 induced by IL6 promotes EGFR-TKI resistance in NSCLC by post-transcriptional repression of active BCR-related protein (ABR) (118). Not only is it IL-6 that can induce EGFR-TKI resistance, but IL-8 can also induce EGFR-TKI resistance by EMT through activation of the MAPK signaling pathway (119).

Integrin-linked kinase (ILK) regulates interactions between tumor cells and the extracellular environment, activating signaling pathways, and promoting cell proliferation, migration, and EMT. SHP2 is critical for the activation of the RTK signaling pathway. Studies have found that ILK and SHP are highly expressed in EGFR-TKI resistant NSCLC (120). Other studies have found that secreted phosphoprotein 1 (SPP1) is significantly increased in EGFR-TKI resistant lung cancer cells, and inhibition of SPP1 increases sensitivity of lung cancer cells

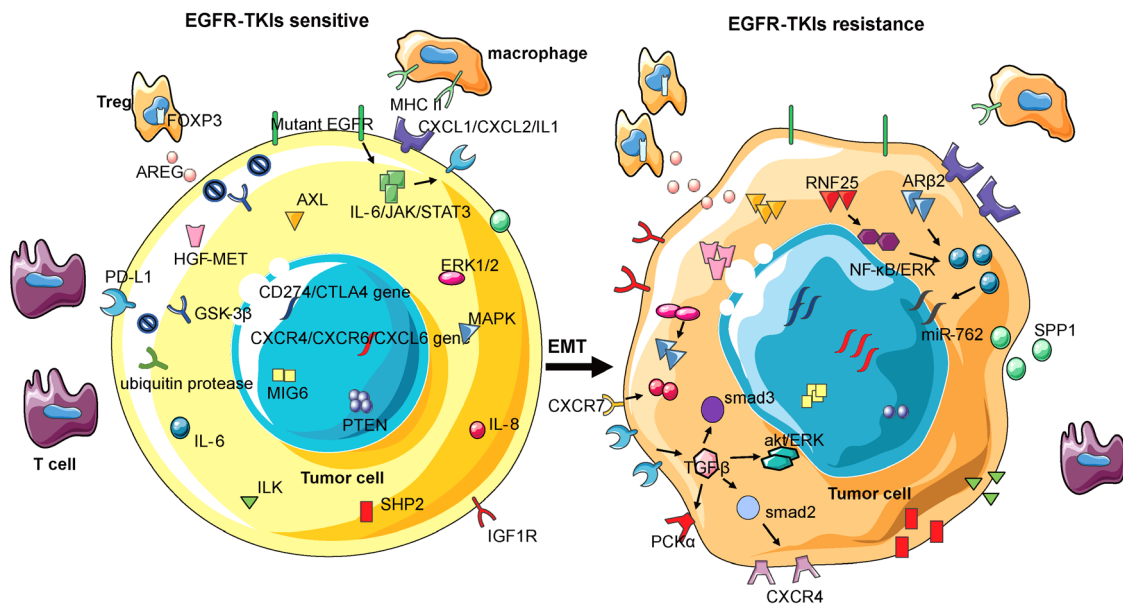


Figure 2 Cytokines in the TIME of EGFR-mutant lung cancer. EGFR-TKIs can induce PD-L1 protein degradation via the GSK3 β and ubiquitin protease pathways, contributing to T cell activation. EGFR mutation can up-regulate amphiregulin (AREG), which maintains Treg suppressive function via the EGFR/GSK-3 β /Foxp3 axis. Axl kinase, genes encoding immune checkpoint molecules (CD274, CTLA4), chemokine receptors (CXCR4, CXCR6), or chemokines (CXCL16) are overexpressed in EGFR-TKI resistant NSCLC. Overexpression of hepatocyte growth factor (HGF) induces EMT via the HGF-MET pathway, resulting in EGFR-TKI resistance. PD-L1 induces EMT via 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-TKI resistance by increasing the expression of PCK α , activation of the Akt-ERK pathway, increasing MIG6 expression, and decreasing PTEN expression. Adrenergic β 2 receptors can induce EGFR-TKI resistance by increasing the expression of IL-6. RNF25 promotes EGFR-TKI resistance by inducing ERK reactivation by the expression of IL6 via the NF- κ B signaling pathway. ILK, SHP, SPP1, and IGF1R are highly expressed in EGFR-TKI-resistant NSCLC. CXCR7 can promote EGFR-TKI-resistance by the EMT of tumor cells through activation of the MAPK-ERK pathway via β -arrestin. TIME, tumor immune microenvironment; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; NSCLC, non-small cell lung cancer; EMT, epithelial mesenchymal transition.

to EGFR-TKIs, decreasing the possibility of invasion (121). Overexpression of type 1 insulin-like growth factor receptor (IGF1R) is associated with EGFR-TKI resistance. Studies have found that overexpression of IGF1R can promote EGFR-TKI resistance by inducing EMT in tumor cells, and inhibition of IGF1R can reverse EMT in tumor cells and sensitivity to EGFR-TKIs (122). CXCR7, an atypical G protein-coupled receptor, can promote EGFR-TKI resistance by the EMT of tumor cells through activation of the MAPK-ERK pathway via β -arrestin (123). Multiple studies have been published in which the relationship between cytokines and EGFR mutation with EGFR-TKI resistance has been investigated, involving complex and diverse signaling pathways, classified generally as tumor EMT. Therefore, inhibitors targeting multiple pathways of

EMT may represent an appropriate research direction for investigating the reversal of EGFR-TKI resistance.

ECM

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 required for tissue morphogenesis, cellular differentiation, and homeostasis (124). Interactions between EGFR-mutated NSCLC tumor cells and the ECM are important in tumor development (Figure 3A). EGFR mutations can increase the expression of twist, snail, and other genes during tumor development, resulting in the induction of epithelial-

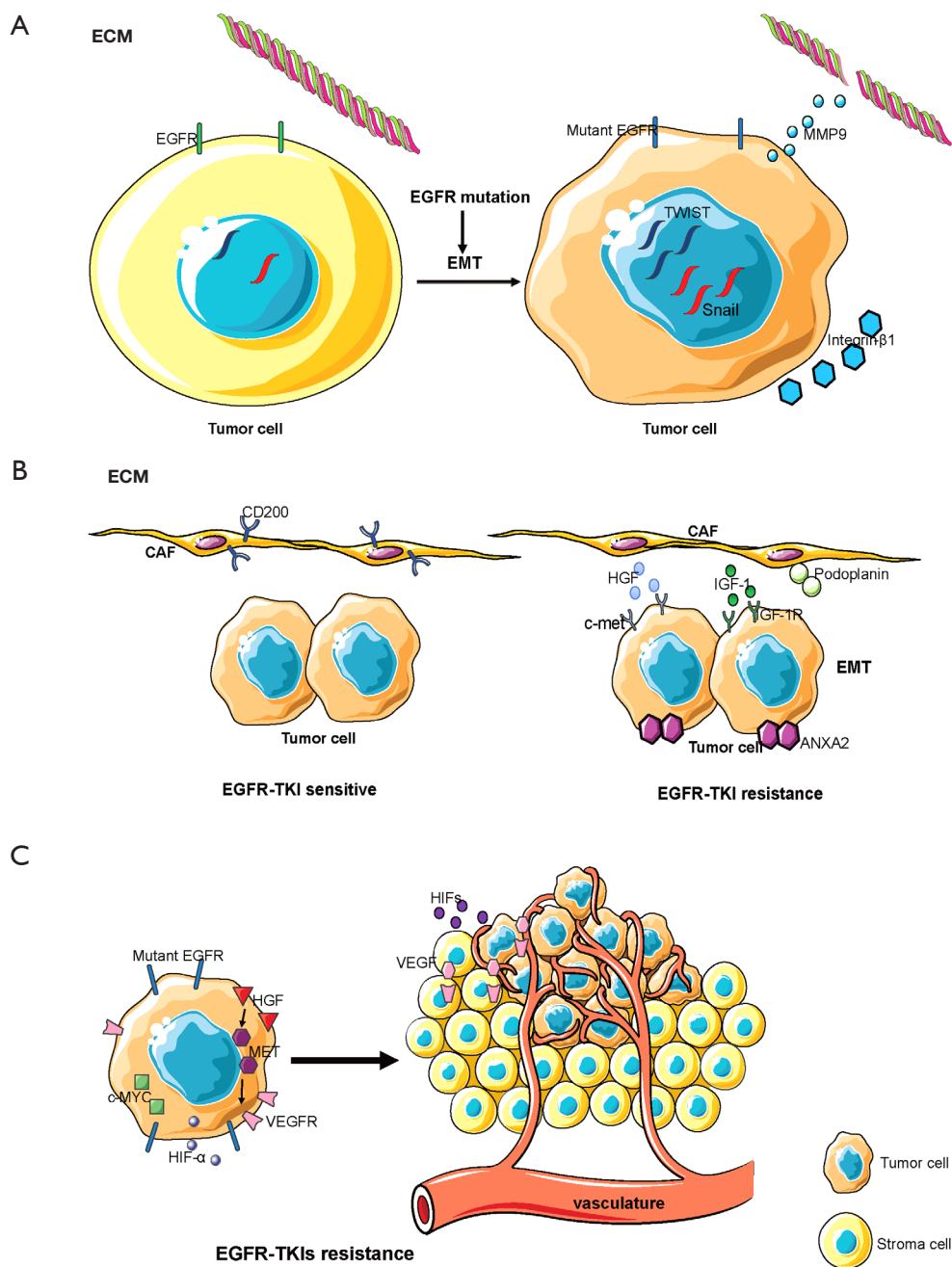


Figure 3 The ECM, CAFs, and vasculature in the TIME of EGFR-mutant lung cancer. (A) The ECM in the TIME of EGFR-mutant lung cancer. EGFR-mutant lung cancer cells overexpress twist and snail, which induces EMT. They also overexpress MMP9, which destroys the ECM barrier. EGFR-TKI-resistant lung cancer cells highly express integrin-β1. (B) CAFs in the TIME of EGFR-mutant lung cancer. In EGFR-TKI-sensitive lung cancer, CAFs highly express CD200. In EGFR-TKI-resistant lung cancer, CAFs highly express HGF and IGF-1, which induce tumor cell overexpression of ANX2 and EMT. They also express podoplanin. (C) The vasculature in the TIME of EGFR-TKI-resistant lung cancer. HGF expression promotes the activation of MET, which stimulates the production of VEGFR. Activation of c-MYC and overexpression of HIF-α also induces EGFR-TKI resistance. ECM, extracellular matrix; CAF, cancer-associated fibroblast; TIME, tumor immune microenvironment; EGFR, epidermal growth factor receptor; EMT, epithelial mesenchymal transition; MMP-9, matrix metalloproteinase-9; HGF, hepatocyte growth factor; IGF-1, type 1 insulin-like growth factor.

mesenchymal transition (EMT) that promotes tumor cell invasion and metastasis. EGFR-TKIs can inhibit EMT and prevent tumor cell invasion and metastasis (125,126). The activation of EGFR in tumor cells also increases the activity of matrix metalloproteinase-9 (MMP-9), which promotes the destruction of the ECM barrier and the invasiveness of tumor cells (127,128). Over recent years, studies have found that the composition of the ECM is associated with EGFR-TKI resistance. The ECM of EGFR-TKI resistant tumor cells have a high expression of integrin- β 1, a collagen receptor, resulting in a large quantity of collagen surrounding tumor cells. EGFR-TKI-sensitive cell lines display EGFR-TKI resistance following their co-culture with integrin β -1 extracted from drug-resistant cell lines, with inhibition of integrin β -1 expression reversing EGFR-TKI resistance (129). The ECM is the location of tumor, stromal, and immune cell interactions. The ECM contains multiple cytokines, proteins, and other soluble substances and so plays an important role in drug resistance. This suggests that there are multiple research avenues regarding the ECM to be studied in the future.

CAFs

CAFs are an important component of the tumor microenvironment and are activated by growth factors, direct intercellular communication, adhesion molecules, and other factors. Dissimilar to normal fibroblasts, CAFs are perpetually activated, do not return to a normal phenotype or undergo apoptosis, and promote tumor progression (130). 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 (130). The function of CAFs in EGFR-TKI sensitive lung cancer is different from that in EGFR-TKI resistant lung cancer (*Figure 3B*). *In vitro* and *in vivo* experiments have demonstrated that the expression and phosphorylation of ANXA2 in CAFs in EGFR-TKI resistant lung cancer are greater due to the secretion of the growth factors HGF and IGF-1, and by activation of the corresponding receptors c-met and IGF-1R, resulting in the induction of EMT in tumor cells and promotion of EGFR-TKI resistance (131). In addition, CD200-positive CAFs in tumor tissues can augment the sensitivity of EGFR-mutant tumor cells to EGFR-TKIs (132). An additional study found that podoplanin-positive CAFs promote EGFR-TKI resistance in tumor cells while inhibition of podoplanin expression in CAFs reversed drug resistance (133). A recent

study found that mesenchymal cells isolated from EGFR-mutant lung cancer can differentiate into CAFs, promoting tumor invasion and metastasis (134). CAFs are therefore associated with EGFR-TKI resistance. Whether inhibition of CAFs can reverse EGFR-TKI resistance requires additional study.

Tumor vasculature

The tumor vasculature is an additional important component of the tumor microenvironment. The vasculature of a tumor arises from two different biological processes: angiogenesis consisting of the formation of new blood vessels from pre-existing vessels and vasculogenesis resulting in the formation of new blood vessels by the recruitment of circulating endothelial progenitor cells (135). Hypoxia inducible factors (HIFs) represent the principal signal regulating the process of angiogenesis which induces the transcription of genes responsible for the activation of angiogenesis (136). Another important regulator is vascular endothelial growth factor (VEGF) and its receptor (VEGFR), which can stimulate angiogenesis (137). The tumor vasculature in EGFR mutant lung cancer is different from that in wild-type EGFR lung cancer (*Figure 3C*). It was found that regions in lung adenocarcinoma with an EGFR mutation that had poor vasculature were smaller than in tumors without the EGFR mutation (138). Another study found that tumors with mutations in exon 20 and 21 of EGFR exhibited high expression levels of VEGFR, while those with mutations in exon 19 of EGFR exhibited low expression levels of VEGFR (139). For EGFR-TKI resistant NSCLC, heat shock protein 90 (Hsp90) inhibitors have been found to overcome HGF-triggered resistance to EGFR-TKIs by reducing EGFR protein expression and tumor angiogenesis (140). Another study found that Met activation by HGF stimulated the production of vascular endothelial growth factor (VEGF) and facilitated angiogenesis, indicating that HGF induces EGFR-TKI resistance and angiogenesis. Triple inhibition of EGFR, Met, and angiogenesis can usefully control the progression of EGFR-mutant lung cancer with HGF-triggered EGFR-TKI resistance (141). *In vivo* and *in vitro* experiments with EGFR-TKIs combined with chemotherapy to treat EGFR-mutant lung cancer found that it inhibited tumor progression and angiogenesis by down-regulation of the c-MYC and HIF- α pathways (142). In addition, a clinical study of VEGFR2 inhibitor combined with EGFR-TKI in the treatment of EGFR mutant NSCLC found that

VEGFR2 inhibitor combined with EGFR-TKI was better than EGFR-TKI monotherapy (143). The formation of a tumor vascular system is associated with EGFR-TKI resistance. Whether antiangiogenic therapy can reverse EGFR-TKI resistance and the associated mechanisms require further investigation.

Conclusions and perspective

In conclusion, the TIME of EGFR-mutated NSCLC is different from that of non-mutated NSCLC, an explanation for EGFR-mutated NSCLC displaying a poor response to ICIs. The TIME of EGFR-mutated NSCLC also changes during treatment with EGFR-TKIs. The TIME in EGFR-TKI-resistant lung cancer can be summarized as follows: (I) compared with EGFR-TKI-sensitive tumors, EGFR-TKI-resistant tumors have a greater number of immunosuppressive cells and fewer immune-activated cells, while the tumor microenvironment is in an immunosuppressive state; (II) tumor cells and immunosuppressive cells secrete multiple negative immune regulatory factors, inhibit the recognition and presentation of tumor antigens and the antitumor effect of immune cells, resulting in immune escape; (III) EGFR-TKI-resistant tumors promote EMT. These three characteristics interact, resulting in a regulatory signaling network, which together leads to EGFR-TKI resistance. Inhibition of one does not fundamentally reverse EGFR-TKI resistance. Therefore, future research on reversal of EGFR-TKI resistance requires the development of pan-targeting drugs that inhibit the key functions of the entire regulatory signaling network, influence immune cell infiltration and immune regulatory factors, reverse EMT, and finally reverse EGFR-TKI resistance.

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