EGFR Mutations and Lung Cancer

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NSCLC: non–small cell lung carcinoma

Mutation: a change in the DNA sequence of a cell's genome that results from substitution, loss, duplication, gain, or abnormal transfer of nucleic acid bases.

Tyrosine kinase (TK): an enzyme that can phosphorylate (i.e., transfer a phosphate group from ATP to) a tyrosine residue in a protein

Predictive markers: clinical, cellular, and molecular markers that predict response of a tumor to treatment; usually assessed by tumor shrinkage or a survival benefit from treatment

TKI: tyrosine kinase inhibitor

RAS: rat sarcoma viral oncogene homolog

RAF: v-raf murine leukemia viral oncogene homolog **MEK**: mitogenactivated protein kinase kinase **MAPK:** mitogenactivated protein kinase PI3K: phosphoinositide 3-kinase

PTEN: phosphatase and tensin homolog **AKT:** v-akt murine thymoma viral oncogene homolog

Abstract

Epidermal growth factor receptor (EGFR) is a transmembrane protein with cytoplasmic kinase activity that transduces important growth factor signaling from the extracellular milieu to the cell. Given that more than 60% of non–small cell lung carcinomas (NSCLCs) express EGFR, EGFR has become an important therapeutic target for the treatment of these tumors. Inhibitors that target the kinase domain of EGFR have been developed and are clinically active. More importantly, such tyrosine kinase inhibitors (TKIs) are especially effective in patients whose tumors harbor activating mutations in the tyrosine kinase domain of the *EGFR* gene. More recent trials have suggested that for advanced NSCLC patients with *EGFR* mutant tumors, initial therapy with a TKI instead of chemotherapy may be the best choice of treatment. Therefore, mutation testing is mandatory to identify these patients, given that selection based only on clinico-pathologic characteristics is inadequate. We review the role of *EGFR* mutations in the diagnosis and management of NSCLC.

Keywords

non–small cell lung carcinoma, epidermal growth factor, receptor tyrosine kinase, tyrosine kinase inhibitor, sensitizing mutation, oncogene addiction

INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality worldwide, with an overall five-year survival rate of 15% . Non–small cell lung carcinoma (NSCLC) constitutes approximately 75–80% of all lung cancers. When the tumor is confined to the lung with minimal regional lymph node spread, the most effective treatment is surgery. However, ~70% of patients present with locally advanced or metastatic disease at the time of diagnosis and are not eligible for surgical resection.

Significant advances in treatment were recently achieved with drugs designed specifically to target molecules that regulate critical growth and/or survival pathways of cancer cells. Dramatic responses in approximately 10% of patients, who had demonstrated resistance to one or more chemotherapy regimens, were reported in Phase I and II trials of the epidermal growth factor receptor (EGFR) tyrosine kinase

inhibitors (TKIs) erlotinib (Tarceva®) and gefitinib (Iressa®). In 2004, the results of the National Cancer Institute of Canada Clinical Trials Group BR.21 study led to the global approval of erlotinib as the first targeted therapy for advanced NSCLC. At approximately the same time, two groups reported the discovery of **mutations** in the tyrosine kinase (TK) domain of the *EGFR* gene in NSCLC. Furthermore, the presence of these mutations appeared to correlate with sensitivity to the EGFR inhibitor gefitinib. Because the patients whose tumors harbored mutations shared certain characteristics with the patients who responded to erlotinib in the BR.21 trial, it was postulated that *EGFR* **TK** domain mutations would be an important molecular **predictive marker** for clinical benefit from EGFR TKIs.

This review summarizes the current knowledge of *EGFR* TK domain mutations and the role they play in the management of NSCLC. It also highlights the importance of *EGFR* mutation status in the selection of patients for EGFR TKI therapy.

EGFR

The *EGFR* gene is located on the short arm of chromosome 7 (7p11.2) and encodes a 170-kDa type I transmembrane growth factor receptor with TK activity. EGFR belongs to the HER/*erb*B family of receptor tyrosine kinases (RTKs), which includes HER1 (EGFR/*erb*B1), HER2 (*neu, erb*B2), HER3 (*erb*B3), and HER4 (*erb*B4). These receptors display similar molecular structures: They have an extracellular, cysteine-rich ligand-binding domain; a single α-helix transmembrane domain; a cytoplasmic TK domain (in all receptors except HER3); and a carboxy-terminal signaling domain. Homodimerization and/or heterodimerization with other family members—most commonly HER2, which lacks its own specific ligand—in response to ligand binding activates the TK.

A ***kinase*** is a type of **enzyme** that transfers **phosphate** groups from **high-energy** donor molecules, such as **ATP** to specific target molecules (substrates); the process is termed ***phosphorylation***. The opposite, an enzyme that removes phosphate groups from targets, is known as a **phosphatase**. Kinase enzymes that specifically phosphorylate tyrosine amino acids are termed **tyrosine kinases**.

This process results in autophosphorylation of the cytoplasmic domain of the receptor and enables it to interact with adaptor molecules, which couple the receptors to downstream signaling pathways. Intracellular signaling is mediated mainly through the **RAS**-**RAF**-**MEK**-**MAPK** pathway, the **PI3K**-**PTEN**-**AKT** pathway, and the signal transducer and activator of transcription (STAT) pathway. Downstream EGFR signaling ultimately leads to increased proliferation, angiogenesis, metastasis, and decreased apoptosis (**Figure 1**).

The TK activity of EGFR may be dysregulated by several oncogenic mechanisms, including *EGFR* gene mutation, increased gene copy number, and EGFR protein overexpression. The receptors and ligands of the EGFR family also mediate complex interactions between tumor cells and the tumor microenvironment. Improper activation of EGFR TK inhibits tumor cell apoptosis and contributes to tumor progression. EGFR may also interact with the integrin pathway and activate matrix metalloproteinases to alter cellular adhesion, stimulate cell motility and invasion, and promote metastasis. Gain-of-function or activating mutations of the *EGFR* gene occur in some **NSCLCs**, leading to

constitutive TK activity. These findings make EGFR a rational target for therapeutic intervention and support the development of novel anticancer agents that target EGFR.

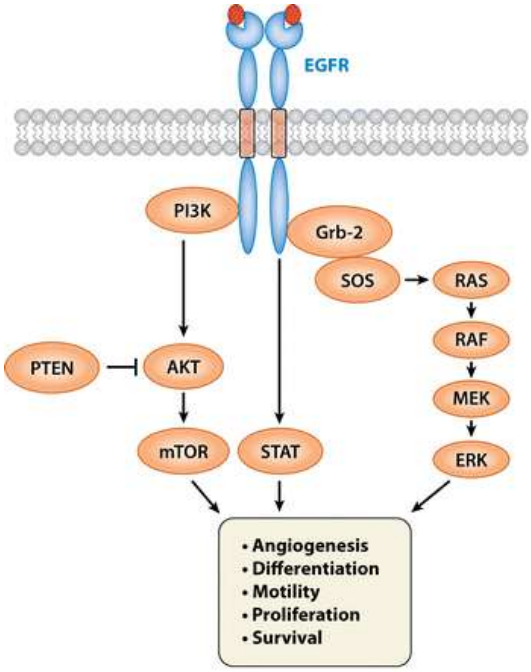


Figure 1. *Simplified schema of epidermal growth factor receptor (EGFR)-induced signals that regulate critical cellular functions relevant to carcinogenesis. Abbreviations: ERK, extracytoplasmic-regulated kinase; Grb-2, growth factor receptor–bound protein 2; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; RAF, v-raf murine leukemia viral oncogene homolog; RAS, rat sarcoma viral oncogene homolog; SOS, sister of sevenless; STAT, signal transducer and activator of transcription.*

EGFR TYROSINE KINASE DOMAIN MUTATIONS

Two independent studies first reported the existence of somatic mutations in the TK domain of EGFR; the **mutations** are characterized by short deletions in **exon** 19 and **point mutations** (G719S, L858R, and L861Q) in exons 19 and 21 (**Figure 2**).

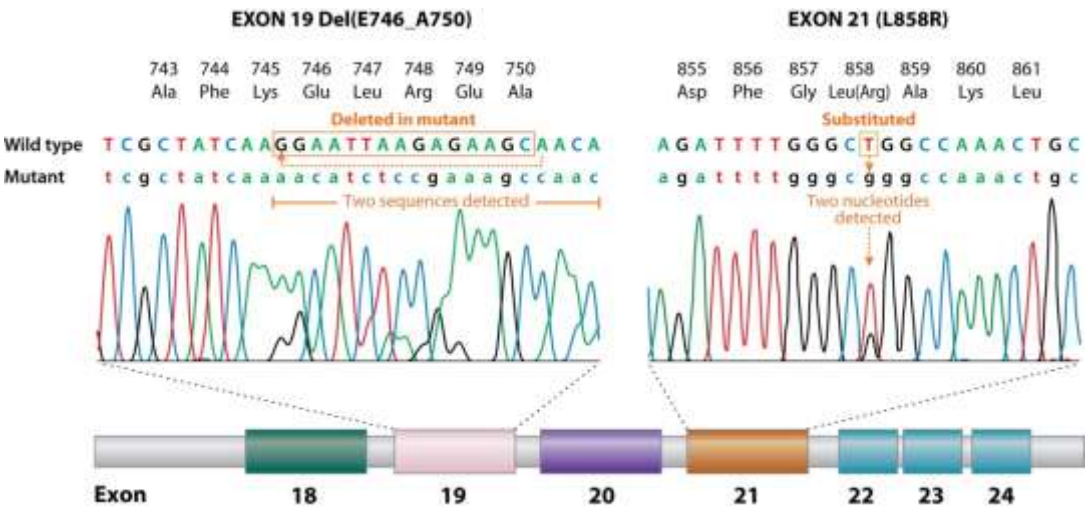


Figure 2. *Amino acid and nucleotide sequence changes in exon 19 deletion and exon 21 L858R mutations involving the tyrosine kinase domain of epidermal growth factor receptor.*

According to their nucleotide changes, the mutations have been classified into three types. Class I mutations include short in-frame deletions that result in the loss of four to six amino acids (E746 to S752) encoded by exon 19. Class II mutations are single-nucleotide

substitutions that may occur throughout exons 18 to 21. Class III mutations are in-frame duplications and/or insertions that occur mostly in exon 20. Among all **TK** domain mutations, 85–90% are exon 19 class I deletions and exon 21 L858R mutations. Although initial reports suggested that there is an almost equal distribution of exon 19 deletions and L858R mutations, more recent reports from clinical trials suggest a slightly higher frequency of deletions versus point mutations. Recently, a rare exon 22 mutation (E884K) that may confer differential sensitivity to different EGFR small-molecule inhibitors was reported.

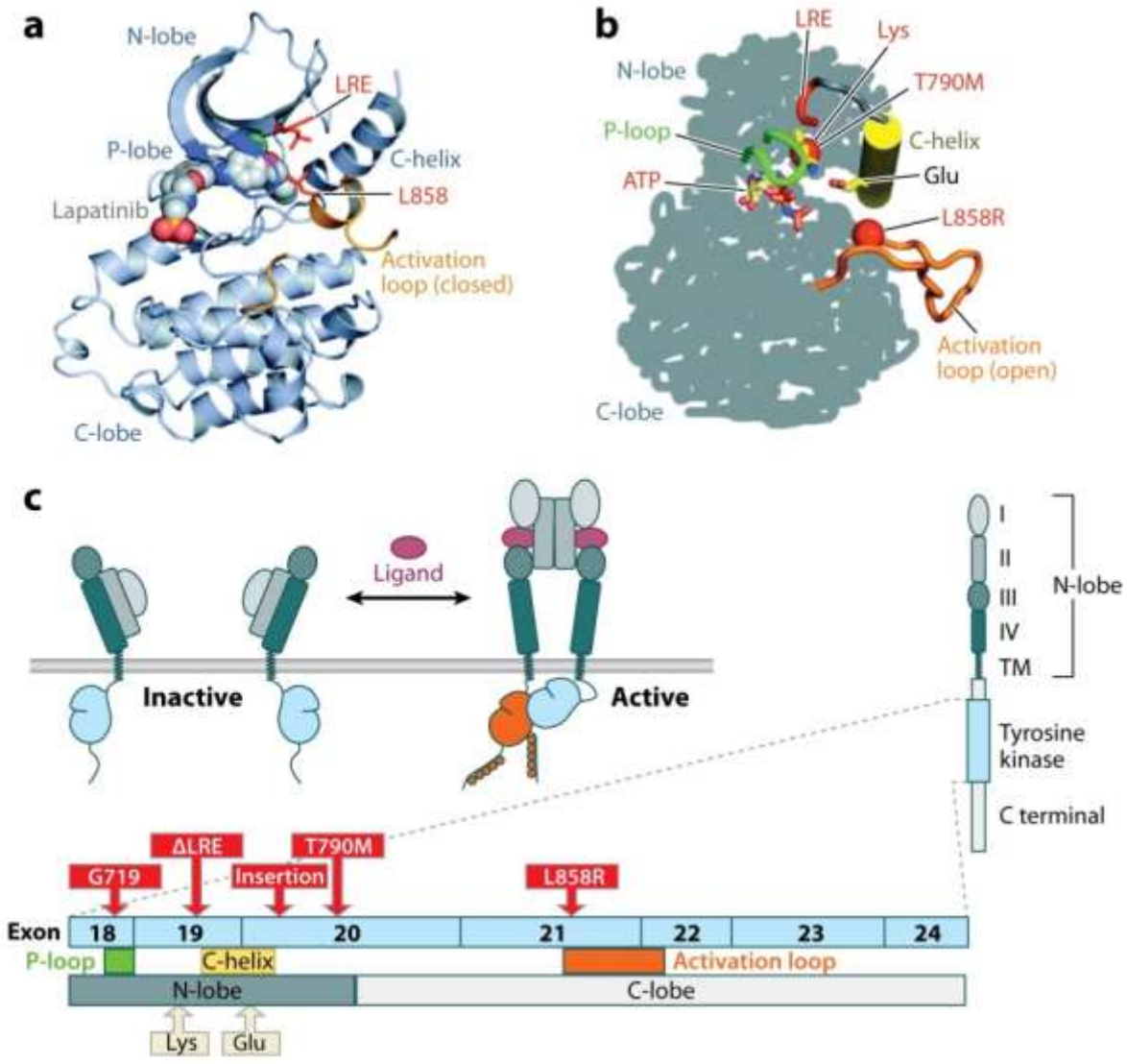


Figure 3. *Structural models reflecting the kinase domain of epidermal growth factor receptor (EGFR) that are (a) wild type and (b) L858R mutant, and (c) locations of identified mutations. (b) In the kinase domain bearing an activated mutant (b), the activation loop is in the open position, which normally occurs only when the receptor is activated by ligand. The ligand-induced asymmetric dimerization of EGFR kinase domain permits head-to-tail interaction between the N-lobe and the C-lobe of the two receptor kinase domains.*

In its inactive form, the EGFR **kinase domain** assumes a structure that results in autoinhibition of its activity. Mutation at the TK domain of *EGFR* results in the destabilization of its domain conformation, constitutive activation of its kinase activity, and activation of its downstream signaling pathways (**Figure 3**) . The latter include AKT and STAT, which have crucial antiapoptosis functions for cell survival . The discovery that lung cancers harboring constitutively active mutant *EGFR* are exquisitely sensitive to the apoptotic or growth inhibitory activities of EGFR **TKIs** strongly supports the so-called oncogene addiction theory, which dominates current views of the role of oncogenic mutations in carcinogenesis and cancer treatment.

PATIENT CHARACTERISTICS ASSOCIATED WITH MUTATIONS

Clinico-pathological features that correlate with *EGFR*-activating mutations include East Asian ethnicity, adenocarcinoma histology, female sex, and a history of never having smoked. Mutations are more common in women than in men (42% versus 14%), in patients who have never smoked than in patients who have smoked (51% versus 10%), and in patients with adenocarcinoma than in those with other histologies (40% versus 3%). Interestingly, the higher mutation rates among patients who have never smoked and adenocarcinoma patients are consistent in both Asian and non-Asian patient populations. The distinct association of mutations with women, patients who have never smoked, and adenocarcinoma patients also suggests different etiologies and mechanisms for the development of NSCLC in Asian and Caucasian patients.

EGFR INHIBITORS

The two major classes of agents designed to inhibit EGFR activity are small-molecule TKIs and monoclonal antibodies. Monoclonal antibodies act by binding to the extracellular region of the receptor and function as competitive antagonists to inhibit ligand binding. Cetuximab (Erbitux®) is a humanized mouse monoclonal antibody developed against the ligand-binding domain of EGFR. Two TKIs, erlotinib and gefitinib, are approved for the treatment of NSCLC. These agents were designed to reversibly bind the ATP-binding site of the EGFR **kinase domain**, thereby inhibiting its activity. Importantly, these agents demonstrate higher binding affinity for EGFR with activating mutation than do the wild-type receptors, consistent with the exquisite sensitivity of the mutant receptor for these drugs.. In contrast to erlotinib or gefitinib, irreversible inhibitors have demonstrated activity against tumors that have developed **secondary resistance mutations**

EGFR MUTATIONS AND RESPONSE TO EGFR TYROSINE KINASE INHIBITORS

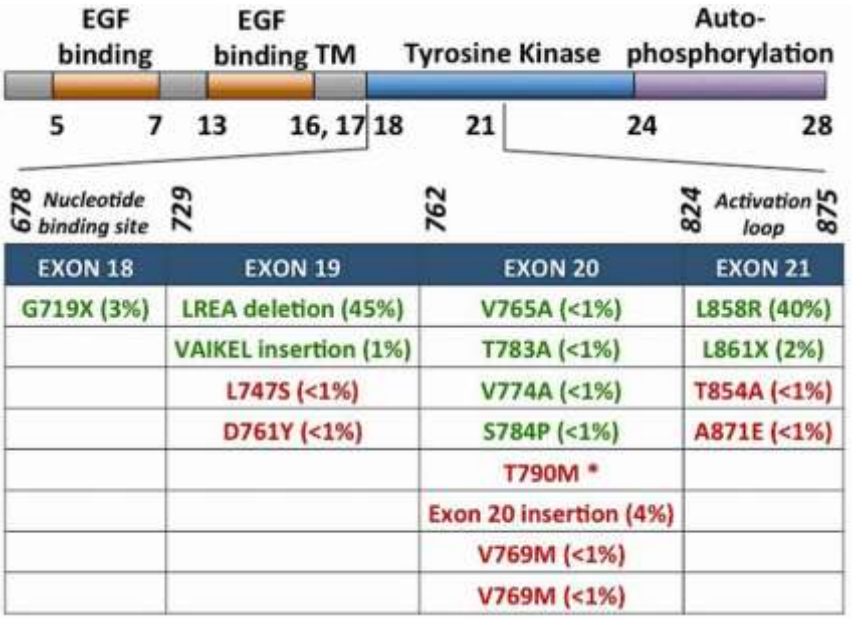
In the original publications reporting the presence of *EGFR* TK domain mutations in **NSCLC**, all 18 refractory patients had wild-type *EGFR* . Since then, the association between the presence of activating *EGFR* mutations and high response rates to TKIs has been confirmed in numerous studies ,which reported overall response rates of 50% to 100% among patients with *EGFR* mutant tumors; response rates among patients with

wild-type *EGFR* are 0% to 30%. The rates are not significantly different between East Asian and Caucasian patients, which suggests that the response is directly related to mutation status and not ethnicity .

Among all *EGFR* mutations, four types are strongly correlated with **TKI** sensitivity in vitro and in vivo. These are point mutations in **exons** 18 (G719A/C) and 21 (L858R and L861Q) and in-frame deletions in exon 19. Mutations in exon 19 of the *EGFR* gene appear to confer sensitivity more often than do **point mutations** in exons 20 and 21. Other mutations may be associated with greater sensitivity to inhibitors, but their rarity precludes any firm conclusions .

EGFR MUTATIONS THAT PREDICT RESISTANCE

Although almost all *EGFR* TK domain mutations that have been detected and studied functionally have been activating mutations, not all of them are associated with increased sensitivity to **TKIs**. Functional studies of one of the exon 20 in-frame insertion mutations using cell-culture models found it to be transforming yet insensitive to the inhibitory effect of gefitinib and erlotinib . Studies on the responsiveness of several class II **point mutations** (E709G, G719S, S768I, and L861Q) found variable responses to the inhibitory activity of gefinitib on both the cellular viability and the EGFR activation of cells expressing these mutants. Only limited information about the clinical response of these rarely reported mutations to EGFR TKI is available. Whereas V689M, N700D, L718P, V765A, V783A, A839T, and K846R are associated with response to gefitinib, E709Q/L, A763V, N826S, and V752I are associated with lack of response .



EXON 18	EXON 19	EXON 20	EXON 21
G719X (3%)	LREA deletion (45%)	V765A (<1%)	L858R (40%)
	VAIKEL insertion (1%)	T783A (<1%)	L861X (2%)
	L747S (<1%)	V774A (<1%)	T854A (<1%)
	D761Y (<1%)	S784P (<1%)	A871E (<1%)
		T790M *	
		Exon 20 insertion (4%)	
		V769M (<1%)	
		V769M (<1%)	

Figure 4. *For summary of somatic mutations found in EGFR. Mutations in green are typically sensitive to EGFR TKIs, those in red are typically resistant. Approximate frequency of occurrence in NSCLC patients of each mutation is shown in parentheses.*

SUMMARY POINTS

1. EGFR is highly expressed in >60% of NSCLCs and plays an important role in regulating the proliferation, survival, motility, and differentiation of the tumor cells.
2. Mutation in the TK domain of *EGFR* results in constitutive and oncogenic activation of the receptor and dependency of the tumor cells in the EGFR signaling pathway.
3. Mutations may occur in many sites on exons 18 to 21 of *EGFR*, but >85% of such mutations consist of short deletions in exon 19 and a L858R point mutation in exon 21. Receptors and cells that harbor these two groups of mutations are highly sensitive to EGFR TKIs.
4. During EGFR TKI treatment of patients whose tumors have sensitizing mutations, disease progression is commonly associated with the identification of additional *EGFR* mutations associated with TKI resistance.
5. *EGFR* mutations define a new group of NSCLCs that can be treated more effectively by specific targeted therapy.
6. Future diagnosis and classification of lung cancer will require incorporation of molecular tests into the standard diagnostic workup.