

The Human Epidermal growth factor Receptor (HER) family: structure and function

Epidermal growth factor receptor (EGFR) belongs to a family of four different receptors, including EGFR (ErbB-1; human epidermal growth factor receptor 1 [HER1]), HER2 (c-ErbB-2), HER3 (c-ErbB-3), and HER4 (c-ErbB-4). These proteins are coded by distinct genes that are expressed on chromosomes 7, 17, 12, and 2, respectively. Although specific soluble ligands have been identified for the extracellular domains of EGFR, HER3, and HER4, no ligand has been identified for HER2. Several ligands can bind to EGFR, including epidermal growth factor (EGF) and transforming growth factor α (TGF α). After the ligand binds the receptor, the receptor dimerizes, either as a homodimer or as a heterodimer preferentially with HER2, but also with other members of the EGFR family, and undergoes autophosphorylation at specific tyrosine residues within the intracellular domain. These autophosphorylation events in turn activate downstream signaling pathways, including the Ras/Raf/mitogen-activated protein kinase (MAPK) pathway, and the phosphatidylinositol 3'-kinase (PI3K)-Akt pathway. Activation of Ras initiates a multistep phosphorylation cascade that leads to the activation of MAPKs. The MAPKs, ERK1, and ERK2 subsequently regulate gene transcription, and have been linked to cell proliferation, survival, and transformation in laboratory studies [1].

Heterodimerization of EGFR and HER2 is an important mechanism of oncogenic transformation in various tumor types. An NIH3T3 cell line

devoid of expression of HER family members was used to evaluate the potency of various HER heterodimers for induction of tumor growth by transfecting various combinations of HER proteins [2]. Cells expressing HER2, HER3, or HER4 homodimers were not able to induce tumor growth, whereas cells expressing EGFR had only modest oncogenic properties. HER2/HER3 coexpression was capable of inducing tumor growth, while combinations of HER1/HER3 and HER1/HER4 did not. Conversely, EGFR/HER2 heterodimers were the only HER dimers inducing an aggressive tumorigenic phenotype (Figure 2.1).

EGFR and HER2 are overexpressed in many solid tumors, including lung, head and neck, breast, kidney, colon, ovary, prostate, brain, and bladder cancers [3–5]. Co-overexpression of EGFR and HER2 potentiates the biologic effect of EGFR and it is associated with the highest

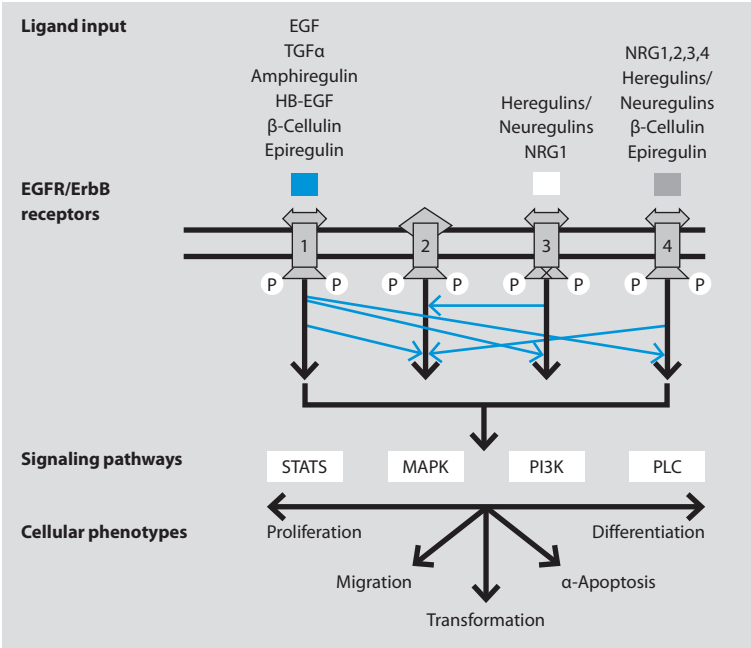


Figure 2.1 The diversity of the EGFR signaling network. ErbB, estrogen receptor (tyrosine kinase family) type B; MAPK, mitogen-activated protein kinase; NRG, neuregulin; PI3K, phosphatidylinositol 3'-kinase; PLC, phospholipase C; STATS, signal transducers and activators of transcription; TGF α , transforming growth factor alpha. Reproduced with permission from © Elsevier Limited 2014, Grandis et al [3].

expression of proliferation markers [6]. Because of its enhanced ability to form heterodimers with other HER family members, HER2 represents the preferred dimerization partner for all of the HER receptors [5]. The mechanism by which HER2 amplifies the mitogenic effect of EGFR is likely heterodimerization [6].

Mechanisms for EGFR deregulation

In non-small cell lung cancer (NSCLC), EGFR is often deregulated. The most frequent events responsible for EGFR deregulation are protein overexpression, gene copy number amplification, or gene mutation (Figure 2.2).

EGFR overexpression

EGFR protein overexpression is seen in up to 85% of patients with NSCLC, although the prognostic relevance of EGFR expression in this disease remains equivocal [7,8]. EGFR expression assessed by immunohistochemistry (IHC) has been the first biological marker to be retrospectively explored in cohorts of patients with NSCLC treated with EGFR tyrosine

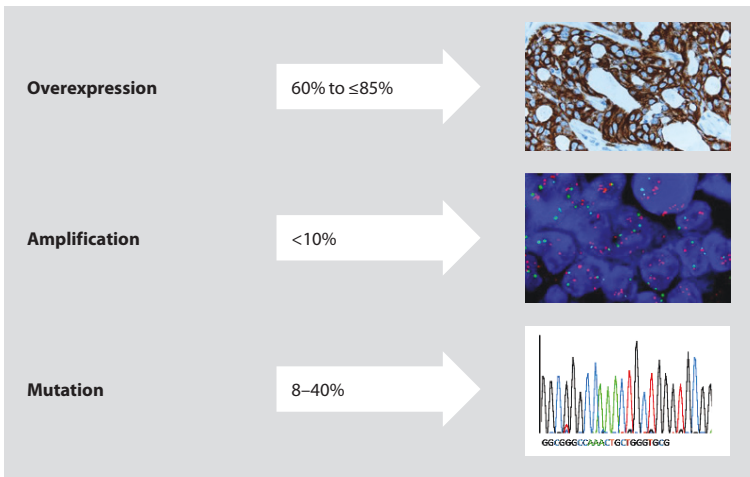


Figure 2.2 EGFR deregulation in patients with lung cancer. The most frequent events responsible for EGFR deregulation are protein overexpression (occurring in up to 85% of cases), *EGFR* gene amplification (reported in up to 10% of patients), and *EGFR* mutations (reported in approximately 10% of Caucasian patients and in up to 40% of Asian patients).

kinase inhibitors (EGFR-TKI). Initial retrospective reports showed no association between EGFR levels and tumor response [9,10]. Two large randomized placebo-controlled phase III trials (BR21 and Iressa Survival Evaluation in Lung Cancer study [ISEL]) evaluated the outcome of patients treated with erlotinib or gefitinib, according to EGFR expression. In the BR21 study [11], 325 tumor samples had undergone IHC staining to assess EGFR protein expression. EGFR-IHC positive patients treated with erlotinib had a significant survival improvement when compared with EGFR-IHC positive patients who received placebo (hazard ratio [HR] 0.68, 95% CI 0.49–0.95, $P=0.02$), while no difference was observed among EGFR-IHC negative patients irrespective of treatment delivered (HR 0.93, 95% CI 0.63–1.36, $P=0.70$). Nevertheless, a formal comparison of these HRs indicated that the difference was not significant ($P=0.25$). Conversely, in the ISEL trial [12], EGFR-IHC positive individuals treated with gefitinib had better survival compared with those treated with placebo (HR=0.77, 95% CI 0.56–1.08, $P=0.126$), with a significant interaction test ($P=0.049$). In the Sequential Tarceva in Unresectable NSCLC (SATURN) trial, a large phase III study comparing erlotinib versus placebo as maintenance therapy in patients not progressing after four cycles of first-line platinum-based chemotherapy, the reduction in risk of disease progression or death was similar with erlotinib irrespective of EGFR expression [13,14].

These data indicate that EGFR expression does not have a consistent predictive or prognostic value across studies for progression-free survival or overall survival. Several factors could explain the weak predictive value of EGFR expression and a recent study highlighted a potential role for EGFR regulators. Zhang et al demonstrated that the multiadaptor protein mitogen-inducible gene 6 (Mig6, also known as ralt, ERRF11, or Gene 33) plays an important role in signal attenuation of the EGFR network by blocking the formation of the activating dimer interface through interaction with the kinase domain of EGFR [15]. More recently, Chang et al demonstrated that tumors not harboring *EGFR* mutations are sensitive to the inhibitory effects of erlotinib in presence of low Mig6/EGFR expression ratio [16], highlighting a potential role for protein expression

evaluated by IHC for the identification of *EGFR* wild-type individuals potentially benefiting from an EGFR-TKI treatment.

***EGFR* gene copy number**

Another mechanism responsible for EGFR deregulation is gene amplification. *EGFR* amplification is reported in approximately 10% of cases with an additional 20% of patients displaying high levels of polysomy [17,18] (Figure 2.3). Different studies evaluated the prognostic impact of *EGFR* gene copy number determined by fluorescence in situ hybridization (FISH), and all showed no association with patient survival [18–21]. In our experience while studying a large cohort of patients with surgically resected NSCLC, no difference in survival was observed in patients

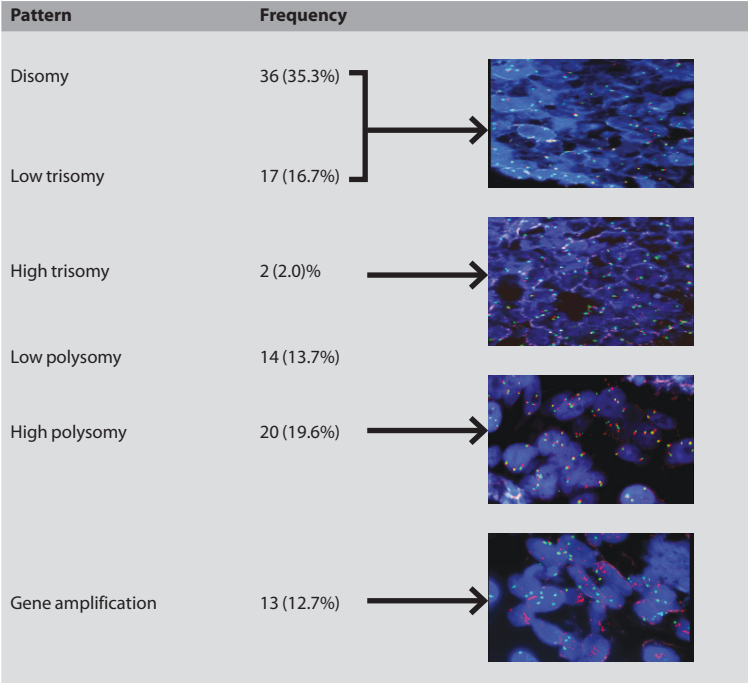


Figure 2.3 *EGFR* gene patterns (fluorescence in situ hybridization). This figure shows EGFR status according to ascending gene copy number. *EGFR* gene amplification is reported in approximately 10% of cases, with additional 20% of patients with high polysomy. Adapted from Cappuzzo et al [17].

with or without gene amplification, nor when the analysis also included individuals with *EGFR* high polysomy [18], as illustrated in Figure 2.4.

Several studies have investigated the predictive implications of *EGFR* gene copy number evaluated by FISH on sensitivity to EGFR-TKIs. In the first study [17], individuals with *EGFR* high polysomy or gene amplification (defined as *EGFR FISH* positive) had a significantly higher response rate, with a significantly longer time to progression and survival than patients with no *EGFR* gene gain (defined as *EGFR FISH* negative). In the randomized placebo-controlled phase III study of erlotinib versus placebo [11], *EGFR FISH* positive patients treated with erlotinib had a higher response rate and longer survival than *EGFR FISH* positive patients treated with placebo (HR 0.44, 95% CI 0.23–0.82, $P=0.008$), whereas erlotinib offered no advantage in *FISH* negative patients. An update of the same

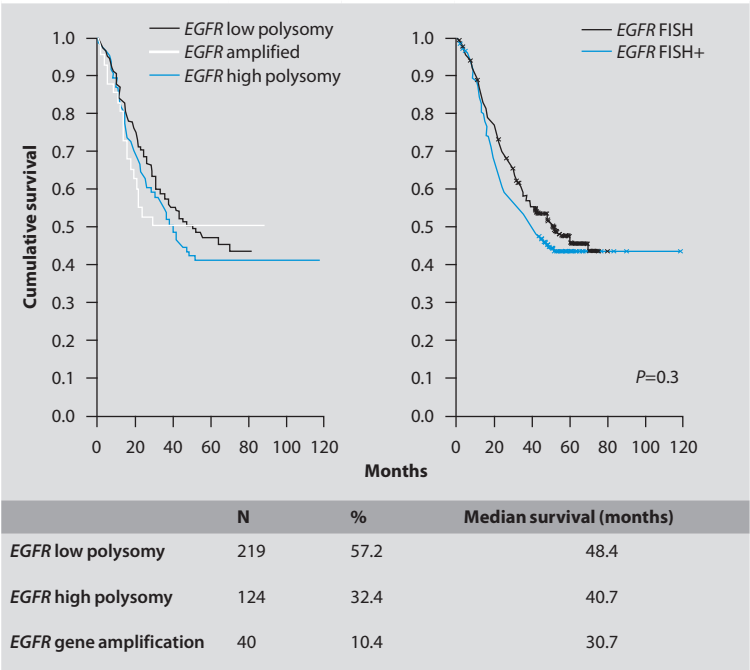


Figure 2.4 *EGFR* copy number and survival in patients with NSCLC. A retrospective analysis of surgically resected NSCLC showed that *EGFR* amplification is not prognostic in resected NSCLC. FISH, fluorescence in situ hybridization. Reproduced with permission from © Cappuzzo et al [18,22].

trial confirmed a significant survival advantage for *EGFR FISH* positive patients treated with erlotinib versus placebo, with a HR of 0.43 [23]. The ISEL trial [12] confirmed the better outcome in terms of response rate and survival for *EGFR FISH* positive patients treated with gefitinib than *EGFR FISH* positive patients treated with placebo, with no survival difference observed between the two arms in *EGFR FISH* patients. The ONCOBELL trial, a prospective phase II study evaluating response rate in *EGFR FISH* positive or never-smoker patients treated with gefitinib confirmed that *EGFR FISH* testing is useful for patient selection [24]. In this study, response rate was 68% in *EGFR FISH* positive and no response was observed in never smokers negative for *EGFR FISH* and mutation.

Nevertheless, two studies mitigated the enthusiasm around FISH testing [25,26]. The Iressa Pan-Asia Study (IPASS) study was a large phase III study that randomly assigned Asian patients with lung adenocarcinoma and who were nonsmokers or former light smokers to front-line therapy with gefitinib or carboplatin plus paclitaxel chemotherapy [27]. In the whole study population gefitinib significantly prolonged progression-free survival versus chemotherapy. Progression-free survival was significantly longer for patients receiving gefitinib whose tumors had both high *EGFR* gene copy number and *EGFR* mutation (HR 0.48, 95% CI 0.34–0.67), but it was significantly shorter when high *EGFR* gene copy number was not accompanied by *EGFR* mutation (HR 3.85, 95% CI 2.09–7.09), indicating that the predictive value of *EGFR* gene copy number was driven by coexisting *EGFR* mutation [25]. The SATURN trial was a large phase III study comparing erlotinib versus placebo in patients with NSCLC not progressing after four cycles of standard first-line platinum-based chemotherapy [13]. The study demonstrated that maintenance erlotinib significantly prolonged progression-free survival and overall survival irrespective of any clinical or biological characteristic, with patients with *EGFR* mutations deriving the highest progression-free survival benefit [13,26]. Erlotinib also produced a significant progression-free survival benefit in patients with *EGFR FISH* positive tumors (HR 0.68, 95% CI 0.51–0.90, $P=0.35$), but no statistically significant benefit in those patients with *EGFR FISH* negative tumors (HR 0.81, 95% CI 0.62–1.07, $P<0.001$) [26]. The interaction between treatment and *EGFR FISH* status

was not significant ($P=0.35$). Overall, available data indicate that *EGFR* gene copy number is not an optimal biomarker for guiding selection of patients with NSCLC for EGFR-TKI therapy.

EGFR mutations

The identification of clinical subsets of patients that are more likely to derive a clinical benefit from gefitinib or erlotinib led investigators to further explore EGFR biology. A milestone in driving future strategies for EGFR-TKI development was realized with the discovery of mutations in the EGFR tyrosine kinase domain of patients responding to EGFR-TKIs [28–30], with preclinical data suggesting that *EGFR* mutant lung cancers are EGFR-addicted for tumor growth [29]. These mutations were somatic and more frequently observed in patients with certain clinical features known to be associated with TKI sensitivity, such as female gender, adenocarcinoma histology, Asian ethnicity, and never smoking history. In addition, Pham et al observed that the likelihood of harboring *EGFR* mutations in lung adenocarcinomas decreases as the exposure to tobacco smoke increases [31]. Mutations were less common in people who smoked for more than 15 pack-years or who stopped smoking cigarettes less than 25 years ago. The most common *EGFR* sensitizing mutations, accounting for approximately 85% of all *EGFR* mutations in NSCLC, include deletions in exon 19 and L858 substitutions in exon 21. Such *EGFR* mutations increase sensitivity to TKIs, most likely through induction of critical structural modifications of the adenosine triphosphate (ATP)-binding site in the tyrosine kinase domain [28,29]. Several other *EGFR* gene mutations have been described but their role is not clear, and it is not possible to exclude the possibility that some of them are artifacts [32]. Rare *EGFR* mutations [33] include insertions in exons 19 (1%) and 20 (4%), point mutations in exon 18 (G719: 3%) and in exon 21 (L861: 2%). The most frequent *EGFR* mutations are located within or are related to the ATP-binding site of the kinase, but rare mutations can be localized in other regions of the tyrosine kinase domain (Figure 2.5). For this reason, the correlation between mutation status and response to EGFR-TKI treatment in patients with rare *EGFR* mutations may be different to that observed in patients harboring classical mutations.

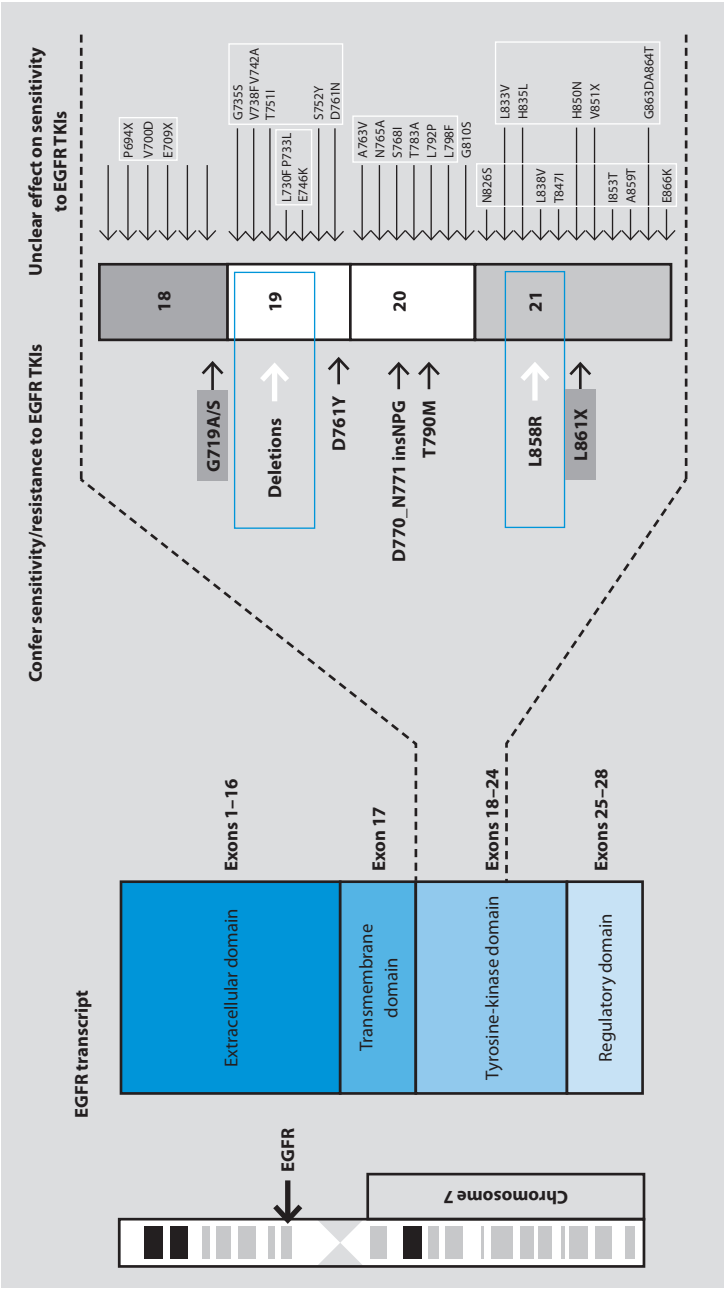


Figure 2.5 Mutations in the EGFR gene. Mutations in exon 19 or in exon 21 are the most frequent EGFR mutations. Additional mutations are reported in other exons. Mutations in exon 20 are generally associated with resistance to gefitinib, erlotinib, or afatinib therapy. TKI, tyrosine kinase inhibitors. Adapted from Riely et al [34].

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Guide to Targeted Therapies: EGFR mutations in NSCLC

Cappuzzo, F.

2014, XI, 67 p. 16 illus. in color., Softcover

ISBN: 978-3-319-03058-6