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Corresponding Author: Jeonghee Cho

Authors: Jeonghee Cho^{1,*}

Institution: ¹Department of Nanobiomedical Science, Dankook University, Chonan, 31116, Republic of Korea,

Mechanistic Insights into Differential Requirement of Receptor Dimerization for Oncogenic Activation of Mutant EGFR and its Clinical Perspective

Jeonghee Cho

Department of Nanobiomedical Science, Dankook University, Cheonan, 31116, Republic of Korea

Abstract

The epidermal growth factor receptor (EGFR), a member of the ErbB family (EGFR, ErbB2, ErbB3 and ErbB4), plays a crucial role in regulating various cellular responses such as proliferation, differentiation, and survival. As a result, aberrant activation of EGFR, mostly mediated through different classes of genomic alterations occurring within *EGFR*, is closely associated with the pathogenesis of numerous human cancers including lung adenocarcinoma, glioblastoma, and colorectal cancer. Thus, specific suppression of oncogenic activity of mutant EGFR with its targeted drugs has been routinely used in the clinic as a very effective anti-cancer strategy in treating a subset of tumors driven by such oncogenic EGFR mutants. However, the clinical efficacy of EGFR-targeted therapy does not last long due to several resistance mechanisms that emerge in the patients following the drug treatment. Thus, there is an urgent need for the development of novel therapeutic tactics specifically targeting mutant EGFR with the focus on the unique biological features of various mutant EGFR. Regarding this

point, our review specifically emphasizes the recent findings about distinct requirements of receptor dimerization and autophosphorylation, which are critical steps for enzymatic activation of EGFR and signaling cascades, respectively, among wildtype and mutant EGFR and further discuss their clinical significance. In addition, the molecular mechanisms regulating EGFR dimerization and enzymatic activity by a key negative feedback inhibitor Mig6 as well as the clinical use for developing potential novel drugs targeting it are described in this review.

Introduction

The ErbB family consists of four members including the epidermal growth factor receptor (EGFR), ErbB2, ErbB3, and ErbB4, and all of which are receptor tyrosine kinases (RTKs) (1, 2). Like the other RTKs, all four members of the ErbB family possess the key structural compositions as follows; extracellular ligand-binding domain, transmembrane domain, intracellular kinase domain, and carboxyl terminal (C-terminal) domain. Previous three-dimensional crystal structural analyses of ErbBs have provided in-depth mechanistic insights into how these domains coordinately contribute to structural rearrangements and overall receptor activation upon ligand stimulation (3, 4). As a consequence of the acquisition of enzymatic activity of the receptor, activated ErbBs initiate phosphorylating of numerous signaling molecules including the receptor itself on tyrosine residues within their C-terminal domains. In turn, these autophosphorylated tyrosine residues of the receptor serve as a docking site for several intracellular adaptor proteins containing SH2, SH3 or PTB motifs, which subsequently trigger a plethora of signaling cascades such as RAS-RAF-MEK-MAPK and PI3K-AKT-mTTO, resulting in key biological effects including proliferation,

migration, differentiation, and survival (5).

Considering the significant role of EGFR as a mitogenic inducer in the cells, it is not surprising that the aberrant activation of EGFR is among the most common oncogenic driving events in human cancer, caused mainly through different classes of genomic alterations within *EGFR* (6). These genomic events include somatic *EGFR* mutations within regions of either the extracellular domain or the kinase domain, as well as through gene amplification as observed in many types of solid tumors (7-10). In addition, several intragenic deletions within either the extracellular or C-terminal domain of EGFR have been identified in glioblastoma and lung adenocarcinoma, and their oncogenic potential was functionally characterized in vitro (11-13).

One key question that has yet to be clearly addressed in the field of EGFR research is whether various oncogenic mutant EGFR are just a functional phenocopy of sustained wildtype EGFR activated by ligands or if these mutant EGFR have unique biological features such as distinct activation mechanisms and/or induction of differential signaling networks. Given that targeting mutant EGFR with specific inhibitors has been used as an effective clinical strategy in treating a subset of tumors harboring such mutations, a detailed elucidation of the biological aspects specific to mutant EGFR would provide invaluable insight on the designing of novel therapeutic tactics.

In this review, we focus on the distinct mechanisms leading to receptor activation between wildtype and mutant EGFR, especially on the requirement of dimerization and also the clinical potential of utilizing this feature in designing novel EGFR-targeted drugs.

1. EGFR activation by receptor dimerization

1.1 Ligand-mediated extracellular domain dimerization

Full enzymatic activation of EGFR is achieved following a series of conformational changes throughout the receptor, initiated by ligand-mediated receptor dimerization. Seven ligands are known to bind to EGFR and the other ErbB family members, which include the epidermal growth factor (EGF), transforming growth factor- α (TNF- α), epiregulin (EPR), heparin-binding EGF-like growth factor (HB-EGF) and amphiregulin (AR) (14, 15). The contribution of these ligands in the rearrangement of extracellular domain priming for EGFR homo- or heterodimerization with the other ErbB family members have been well characterized by numerous crystal structure studies (16-19). The extracellular regions of EGFR comprise two homologous ligand binding domains (domains I and III) and two cystine rich domains (domains II and IV). In the absence of ligands, EGFR exists as an inactive tethered conformation in which domains II and IV interact and occlude domain II “dimerization arm” involved in receptor dimerization. Notably, based on the current proposed model, this tethered state is preferred in the absence of ligands, but it is not the only form and multiple untethered conformations could exist in a dynamic equilibrium. The binding of ligands to domain I and III stabilizes the un-tethered receptors in a dimerization-competent extended conformation, consequently driving the overall equilibrium shift from an inactive tethered monomer towards the active dimeric forms (20) (Figure 1A). Thus, it

is well accepted that the stabilization of dimerization arm exposed monomeric forms led by various ligand-mediated extracellular domain rearrangement could be a crucial step leading to receptor dimerization.

1.2 Asymmetric dimerization of kinase domains

There is no doubt that one of the breakthrough studies in the field of EGFR biology is the detailed crystal structure-based mechanistic elucidation of asymmetric dimerization of kinase domains-mediated EGFR activation (21, 22). This study revealed that two interfaces between the N-lobe of one kinase domain, termed “receiver”, and C-lobe of the other kinase domain, termed “activator”, interact in an asymmetric manner. Consequently, the activator monomer induces the conformational changes of the N-lobe of the receiver monomer, similar to cyclin-induced activation of cyclin-dependent kinases, resulting in the enzymatic activation of the receiver monomer in a dimer (22, 23) (Figure 1A). The functional significance of this finding was further validated by several following reports (24, 25). For example, it was shown that the disruption of asymmetric dimerization through substitution mutations at the dimerization interface, such as L704N (receiver-impairing mutation) in the N-lobe and I941R (activator-impairing mutation) in the C-lobe, abolished ligand-induced EGFR activation and consequent cellular transformation (22, 24, 25). In addition, the co-expression of receiver-impaired and activator-impaired EGFR mutants can rescue EGFR activation through a subset of reestablished asymmetric dimerization between the intact C-lobe and the intact N-lobe of the respective EGFR mutants (25). Also, compelling evidence from numerous functional and structural studies support the

current model that enzymatic activation of EGFR is mediated by the ligand-mediated extracellular dimerization of EGFR followed by the asymmetric dimerization of the kinase domains of two monomers in an allosteric manner (24-27). Thus, the coordination of two distinct dimerization allosterically achieved through conformational changes of the receptor may function as critical steps in inducing enzymatic activation of EGFR. Notably, several regulatory mechanisms tightly modulating these processes have been identified as essential cellular devices to control receptor activation as well as to prevent unnecessary EGFR activation.

2. Genomic alterations of EGFR and mechanistic insights into oncogenic activation

2.1 Various types of EGFR mutations in cancer

Several different classes of genomic mutations within *EGFR* have been found in various cancer types, including lung adenocarcinoma, glioblastoma (GBM), and colorectal adenocarcinoma (7, 12, 24, 28-30) (Figure 2). In addition, numerous structural and functional studies have shown that a subset of patient-derived EGFR mutations are directly associated with ligand-independent receptor activation and dysregulation of the EGFR signaling cascade, consequently resulting in cellular transformation (31-33). In lung adenocarcinoma, the most dominant mutations are within the kinase domain of EGFR, including L858R in exon 21 and various small in-frame deletions in exon 19 (Ex19Del), with these genomic alterations making up ~ 90% of EGFR mutations identified from lung adenocarcinoma (34) (Figure 2). In particular,

these two prevalent types of somatic mutations have clinical significance because lung tumors harboring such mutations are highly sensitive to EGFR kinase targeted inhibitors such as gefitinib and erlotinib (35). Thus, the EGFR mutation status in lung cancer patients serves as a crucial genomic determinant and biomarker for clinical outcomes with EGFR-directed therapy. In GBM, *EGFR* alterations were frequently identified as either intragenic deletions between exons 2-7 (denoted as EGFR vIII), exons 14-15 (denoted as EGFRvII) or somatic alterations within the extracellular domain of the receptor, but lung cancer prevalent kinase domain mutations are relatively rare (7, 36) (Figure 2). Furthermore, recent genomic studies identified several additional intragenic deletions of exons encoding the EGFR C-terminal domain in GBM, also found in lung adenocarcinoma (7, 12) (Figure 2). Subsequent studies have showed that these resulting C-terminal truncation mutants retain the ability to induce oncogenic transformation and tumorigenesis (12, 27). Notably, *in vitro* and *in vivo* experiments clearly demonstrated that therapeutic anti-EGFR monoclonal antibodies such as cetuximab are highly effective against GBM-derived C-terminal deletion EGFR variants (12, 27). However, the clinical efficacy of cetuximab-directed therapy for GBM patients harboring such mutations has not been proven. In colorectal cancer, according to the public genomic databases (e.g. cBioportal), EGFR mutations have been known to be very rare (~3%). However, very recent systematic functional and biochemical studies with recurrent EGFR mutations selected from public genomic datasets of colorectal cancer revealed that a subset of colon cancer-derived EGFR mutants function as a strong oncogenic driver in a ligand-independent manner (24, 26) (Figure 2). This result suggests that although EGFR mutations are not as common as other cancer types, these somatic alterations may contribute to the pathogenesis of

colorectal cancer. Significantly, the oncogenic potential of these EGFR mutants is efficiently inhibited by cetuximab or panitumumab *in vivo* and *in vitro* (24, 26). Thus, further exploration is needed as to if these EGFR mutations can serve as clinically beneficial genomic biomarkers for anti-EGFR antibodies in colon cancer patients with such mutations as shown in lung adenocarcinoma.

2.2 Distinct requirement of dimerization for oncogenic activation of mutant EGFR

It is well characterized that various EGFR oncogenic mutations induce constitutive activation of enzymes that are not dependent on ligands (12, 24, 31). Given that asymmetric dimerization of kinase domains directed by ligand-mediated extracellular dimerization is a critical step for canonical enzymatic activation of wild-type EGFR (22), it is of significance to examine whether ligand-independent EGFR mutants still require asymmetric dimerization for constitutive receptor activation leading to oncogenic cellular transformation. Several biochemical and functional studies have been reported regarding these issues. A key experimental strategy used in these studies was to functionally evaluate the dependency of dimerization in the oncogenic potential of various cancer-derived mutant EGFR by characterizing the dimerization-disrupted compound EGFR mutants with either a receiver-impairing mutation (L704N) and/or an activator-impairing mutation (I941R) using NIH-3T3 cells, which express low to undetectable levels of endogenous EGFR. These dimerization-incompetent EGFR mutant-expressing cells were then assayed for enzymatic activity of EGFR mutants and their ability to grow in soft agar. In this system, the EGFR

activation and transforming ability of dimerization-dependent mutants is predicted to be abolished by *cis* mutation of L704 or I941 (22). Thus, this experiment facilitates determining whether various EGFR mutants can be activated and induce cellular transformation in a dimerization-dependent or -independent fashion. Based on the results from these approaches, interestingly, two distinct requirements of dimerization for oncogenic activation were shown to exist among various mutant EGFR. While the lung cancer-derived L858R, GBM-derived C-terminal deletions and most of colon cancer-derived EGFR mutants require dimerization for their oncogenic activation (Figure 1B), a subset of lung-cancer derived mutants such as Ex19Del, Ex20Ins, and L858R/T790M EGFR mutants do not depend on dimerization for constitutive receptor activation (24-27) (Figure 1C). These results suggest that a key molecular mechanism leading to oncogenic activation of L858R, C-terminal deletions and colon cancer-derived EGFR mutants may be linked to the acquisition of the ability to form ligand-independent constitutive dimerization on the cell surface. In contrast, the other group including Ex19Del, Ex20Ins, and L858R/T790M EGFR mutants appears to acquire oncogenic activation irrespective of these dimerization (25). Thus, two distinct classes of mutant EGFR exist based on the dependence on dimerization for oncogenic activation. Also, it has been shown that the difference in dimerization requirement among mutant EGFR is also associated with the induction of various downstream signaling networks leading to cellular transformation. For example, the addition of dimerization-impairing *cis* mutations, L704N and I941R, in the dimerization-dependent EGFR mutants significantly reduced their ability to promote colony formation of NIH-3T3 cells upon retroviral transduction (24-27). In contrast, introduction of the same dimerization-disruption mutations into the dimerization-independent group of EGFR

mutants had little effect on their ability to induce colony formation under the same experimental condition, suggesting that this group of mutants can indeed result in cellular transformation irrespective of dimerization (25).

Notably, these unexpected results are further supported by the recent report showing that C-terminal autophosphorylation of dimerization-independent mutant EGFR such as Ex20Ins is not required for oncogenic transformation (37). One proposed explanation for this result is that Gab1/2, Shc1 and Bcr1 adaptors may interact in a mutant-EGFR-specific manner and function as crucial factors in mediating constitutive oncogenic activation of various signaling pathways independently of asymmetric dimerization and C-terminal phosphorylation (37).

In summary, EGFR mutants seem to become active via different paths, which can be achieved in a dimerization-independent manner and also lead to activation of signaling networks without C-terminal autophosphorylation. The more detailed molecular mechanisms and its functional significance of these non-canonical biological features of mutant EGFR need to be further explored in future studies.

3. Regulation of EGFR dimerization as negative feedback mechanism

3.1 Key effectors associated with negative regulation of active EGFR

Deactivation of ligand-stimulated active EGFR is temporally and spatially regulated via numerous mechanisms to prevent unnecessarily sustained cellular responses, closely associated with the pathogenesis of various diseases including cancer. These negative regulatory mechanisms of EGFR can be classified into two

major modes of action which includes degradation of ligand-bound active EGF receptors, and inactivation of enzymatic activity of the receptor (38). Collectively, these processes are mediated by tightly coordinated actions of a plethora of proteins which effectively impede the function of active EGFR as well as its downstream signaling cascade. Specifically, the degradation of active EGFR is primed via the ubiquitination of the receptor resulting from the direct binding of Cbl, E3 ubiquitin ligase, to autophosphorylated EGFR at tyrosine 1069, which subsequently induce the sorting of the receptor to lysosomal fusion for its degradation (39). In addition, four negative feedback inhibitors, LRIG, Mig6 (also called RALT) and SOCS4/5, transcriptionally induced by activated ErbB signaling networks, have been identified as the key regulators of ErbB activity including EGFR (40). Among them, the functional roles of Mig6 as a negative effector of EGFR are the most intensively characterized (41) and the molecular mechanisms underlying Mig6-mediated negative regulation of EGFR is specifically discussed below based on recent findings from several structural and functional studies.

3.2 Mig6 functions as a key negative regulator of EGFR

Mitogen-inducible gene 6 (Mig6), also known as RALT, is a negative feedback inhibitor of EGFR and other ErbB family members. Mig6 expression is transcriptionally induced by hormones, growth factors, and various stress stimuli via the Ras-Raf-ERK signaling pathway (42, 43). By complexing with EGFR mediating revolutionary conserved domains (segment 1 and 2) (44), Mig6 effectively inhibits EGFR activation and its downstream signaling pathways via three proposed mechanisms in an

orchestrated manner. First, Mig6 binds to the C- lobe of the active EGFR kinase domain through segment 1 and blocks the asymmetric dimer formation with the N-lobe of the other EGFR monomer, required for full enzymatic activation of EGFR as described above (45) (Figure 3). Second, phosphorylated Mig6 at segment 2 binds strongly to the EGFR kinase domain and directly suppresses its catalytic activity in a peptide substrate competitive manner (46) (Figure 3). In addition, Mig6 can complex with EGFR and induce its internalization and degradation (47, 48). Such coordinated actions seem to render the Mig6 as a tight negative regulator of EGFR and its signaling circuits to prevent aberrant EGFR activation leading to cellular transformation. Consistent with this notion, the loss of *Errfi1* (ERBB receptor feedback inhibitor 1, official gene name of Mig6) in mice was shown to induce a high incidence of neoplastic lesions caused by abnormal activation of EGFR signaling (49). Furthermore, frequent focal deletions of *ERRFI1* were identified from the analysis of 1,057 gliomas supporting the fact that the loss of Mig6 is a key driving force of human cancer (46).

4. Clinical significance of blockage of EGFR dimerization

4.1 EGFR targeted monoclonal antibody

Several EGFR-directed therapeutic monoclonal antibodies (mAb) such as cetuximab and panitumumab are effective in the treatment of a subset of tumors such as colon cancer (50, 51). Although it is unclear, previous structural studies suggested that one proposed pharmacological mode of action of these EGFR-targeted Abs is to interfere with EGFR dimerization via specifically binding to the ligand interacting region of the receptor so that the drugs block the enzymatic activation of EGFR (20). As

discussed above, given that a subset of oncogenic EGFR mutants such as exon 19 deletion and exon 20 insertion mutants are oncogenically active irrespective of receptor dimerization, it is postulated that antibodies directed at the “upstream” extracellular domain may be ineffective against these dimerization-independent oncogenic mutant EGFR. Several recent *in vivo* and *in vitro* studies provided compelling evidence to support this hypothesis, showing that there is a close relationship between dimerization dependency and EGFR-directed mAb responses among mutant EGFR (24-27). For example, mouse lung tumors formed by dimerization-dependent L858R and G719S EGFR mutants are dramatically reduced by cetuximab treatment, whereas tumors induced by dimerization-independent mutant EGFR such as the exon 20 insertion mutant are resistant to cetuximab (25) (Figure 1B, 1C and Figure 2). Similarly, the oncogenic activity of GBM-derived C-terminal deletion mutants, caused by their constitutive dimerization, is efficiently inhibited by cetuximab *in vivo* and *in vitro* (27). Furthermore, recent reports demonstrated that all dimerization-dependent colorectal cancer-derived EGFR have a dramatic response to cetuximab and panitumumab (24, 26) (Figure 1B and Figure 2). Taken together, these data suggest that ligand-independent constitutive receptor dimerization caused by somatic mutations within *EGFR* is a key molecular mechanism leading to oncogenic activation of EGFR (52) and disruption of dimerization may be among the pharmacological mechanisms of EGFR-targeted mAbs. Thus, it was proposed that the requirement of dimerization for oncogenic activation among mutant EGFR may be a crucial predictive factor of clinical response to cetuximab as a close correlation exists between dimerization dependency and its pharmacological effects. This hypothesis needs to be further explored in future studies in clinical settings.

4.2 Mig6-based dimerization inhibition

The breakthrough findings of the key molecular mechanism underlying Mig6-mediated negative feedback regulation of EGFR was conducted through X-ray crystal-based structural studies followed by elaborate biochemical characterization (45). These studies revealed that Mig6 strongly interacts with EGFR in the asymmetric dimerization interface of EGFR kinase domain C-lobe and N-lobe regions. Also, this interaction is mediated by a specific domain of Mig6 known as segment 1 (S1), one of two evolutionary conserved regions within Mig6 (45). Given that EGFR asymmetric dimerization is required for full enzymatic activation of EGFR (22), the binding of Mig6 S1 to EGFR abrogates the formation of intact dimerization of EGFR and consequently blocks its activation (Figure 3). Mig6 also appears to prevent asymmetric dimerization among the other ErbB family members via the same interacting mechanism (45). Thus, the preclusion of asymmetric dimerization by Mig6 S1 is a key negative regulatory mechanism among ErbB members.

One puzzling observation not clearly addressed in the original structural analysis was the functional significance of the other interaction of Mig6 at the substrate binding site of EGFR, mediated by Mig6 segment 2 (S2) (45). Recent additional crystal structural, biochemical and functional studies demonstrated the role of Mig6 S2-mediated interaction in inhibiting EGFR activation and identified the detailed molecular mechanism of how Mig6 S2 contributes to this process (46, 53). According to these findings, Mig6 remains an incomplete inhibitor of EGFR until a series of posttranslational modifications occurs in segment 2; Mig6 undergoes tyrosine

phosphorylation at 395 in S2 region by Src, which in turn becomes a suitable substrate of EGFR (46). Next, additional phosphorylation is induced by EGFR in Mig6 at tyrosine 394, which dramatically increases the binding affinity of Mig6 to the EGFR active site and consequently, Mig6 becomes a potential competitive inhibitor blocking other substrate access to EGFR (46) (Figure 3). The functional significance of these events was further validated using *in vitro* models. While phosphorylation-competent Mig6 effectively prevents EGFR mutants from causing oncogenic transformations, this was not the case with Mig6 Y394F/Y395F mutants under the same experimental condition (46).

In conclusion, Mig6 negatively regulates EGFR and its signaling pathways through several distinct mechanisms; blockage of EGFR asymmetric dimerization by the S1 region and potential competitive inhibition of substrate access by the phosphorylated S2 region. This signaling circuit-based regulatory mechanism renders Mig6 effective in selectively targeting active EGFR.

Perspective

Despite the proven effectiveness of EGFR-directed therapy in treating a subset of tumors driven by aberrant EGFR, its clinical efficacy is limited due to various resistance mechanisms such as additional T790M secondary mutation within *EGFR* emerging among patients following treatment of these drugs (54). Currently, numerous efforts have been made to generate additional effective targeted drugs to overcome such resistance and a number of FDA-approved drugs are already used in clinics (55). However, most next generation drugs are also based on the ATP-analogs and thus remain highly susceptible to developing inevitable resistance to them (56).

Considering that dimerization of EGFR is a critical biological step required for its enzymatic activation, an ideal approach may be to take advantage of these features in developing novel EGFR-targeted drugs (57). Regarding this point, Mig6 segment 1-based peptides specifically targeting dimers can be considered as a novel potential drug particularly for dimerization-dependent mutant EGFR. In addition, given that Mig6 can effectively block the oncogenic activity of most mutant EGFR including T790M mutant (46), a Mig6 394/395 phospho-peptides based drug design can also be adopted as an attractive approach to overcome current drug resistance. Several recent reports showed that small peptides encompassing Y394/Y395 can effectively bind to EGFR and block the enzymatic activity of EGFR (58, 59). The clinical significance of these peptides as a potential EGFR-directed drug need to be further investigated in the future.

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Differential requirement of EGFR dimerization for activation

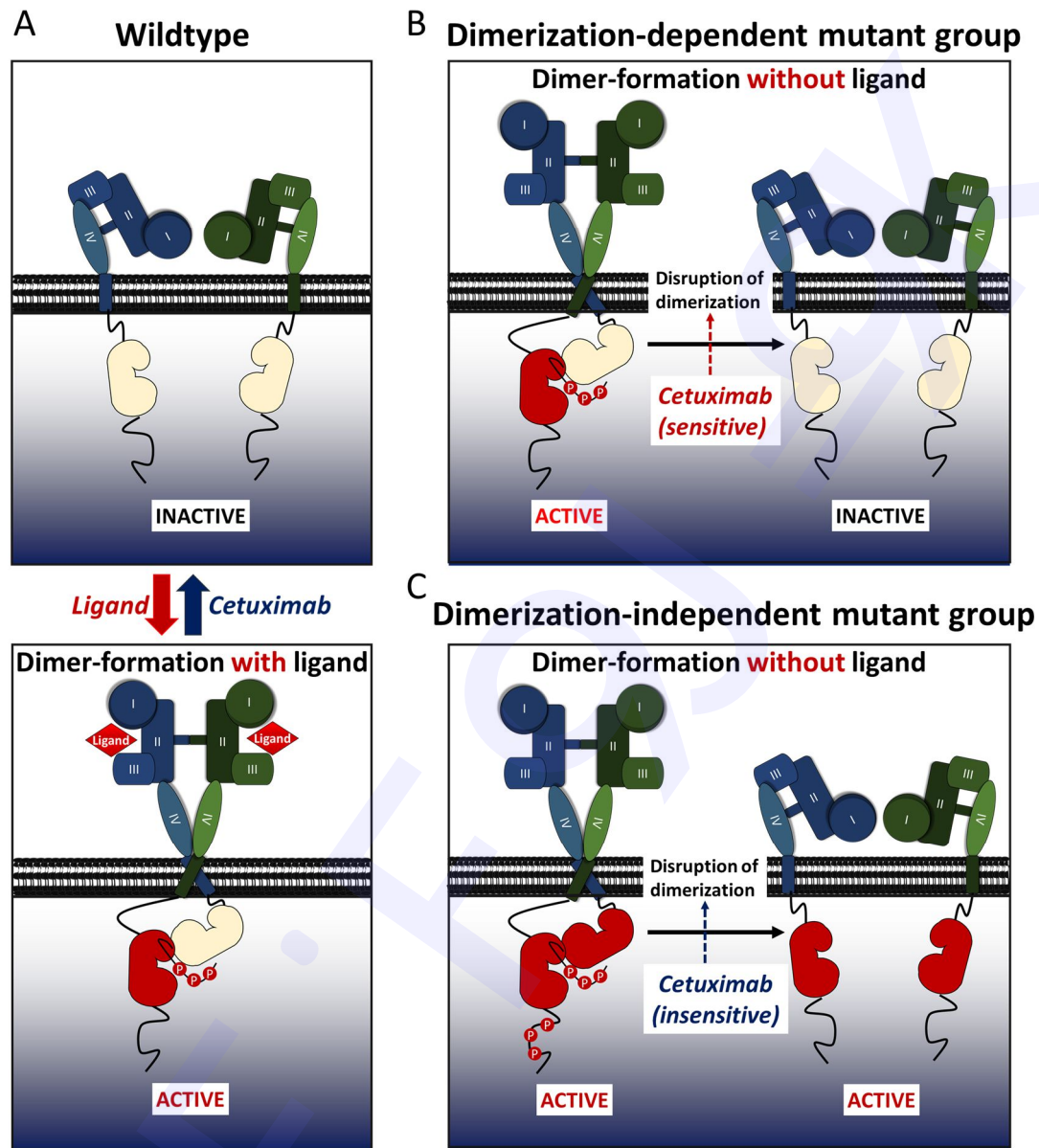


Figure 1. Proposed model of dimerization requirements for receptor activation by wildtype EGFR and oncogenic mutants (adapted from Cho et al., 2013).

(A) Activation of wildtype EGFR is initiated by ligand-induced extracellular dimerization of the receptor, which in turn direct “outside-in” asymmetric dimerization of the N- and C-lobes in the kinase domain, leading to enzymatic activation of the receiver monomer and subsequent autophosphorylation on the C-terminal tail of the activator monomer. Therefore, full enzymatic activation and consequent signaling cascade of the wildtype EGFR are tightly dependent on all sequential events of ligand binding, dimerization and autophosphorylation. (B) A group of EGFR mutants including the L858R, most colon cancer-derived and C-terminal truncation EGFR mutants are able to form

constitutive asymmetric dimerization irrespective of ligand binding, which in turn promotes “inside-out” extracellular dimerization; ligand increases dimerization but is not required. Interruption of dimerization impairs the oncogenic activity of these types of mutant EGFR because the receiver monomer is no longer active. Thus, mutant EGFR belong to this group is sensitive to EGFR-directed mAbs such as cetuximab. (C) In contrast, a subset of mutant EGFR including the Ex19Del and Ex20Ins mutants and the L858R/T790M double mutants similarly undergo dimerization in the absence of ligand, but do not require dimerization for their activity. These mutant forms of EGFR acquire the oncogenic activity in dimerization-independent manner so that these mutants are refractory to EGFR mAbs.

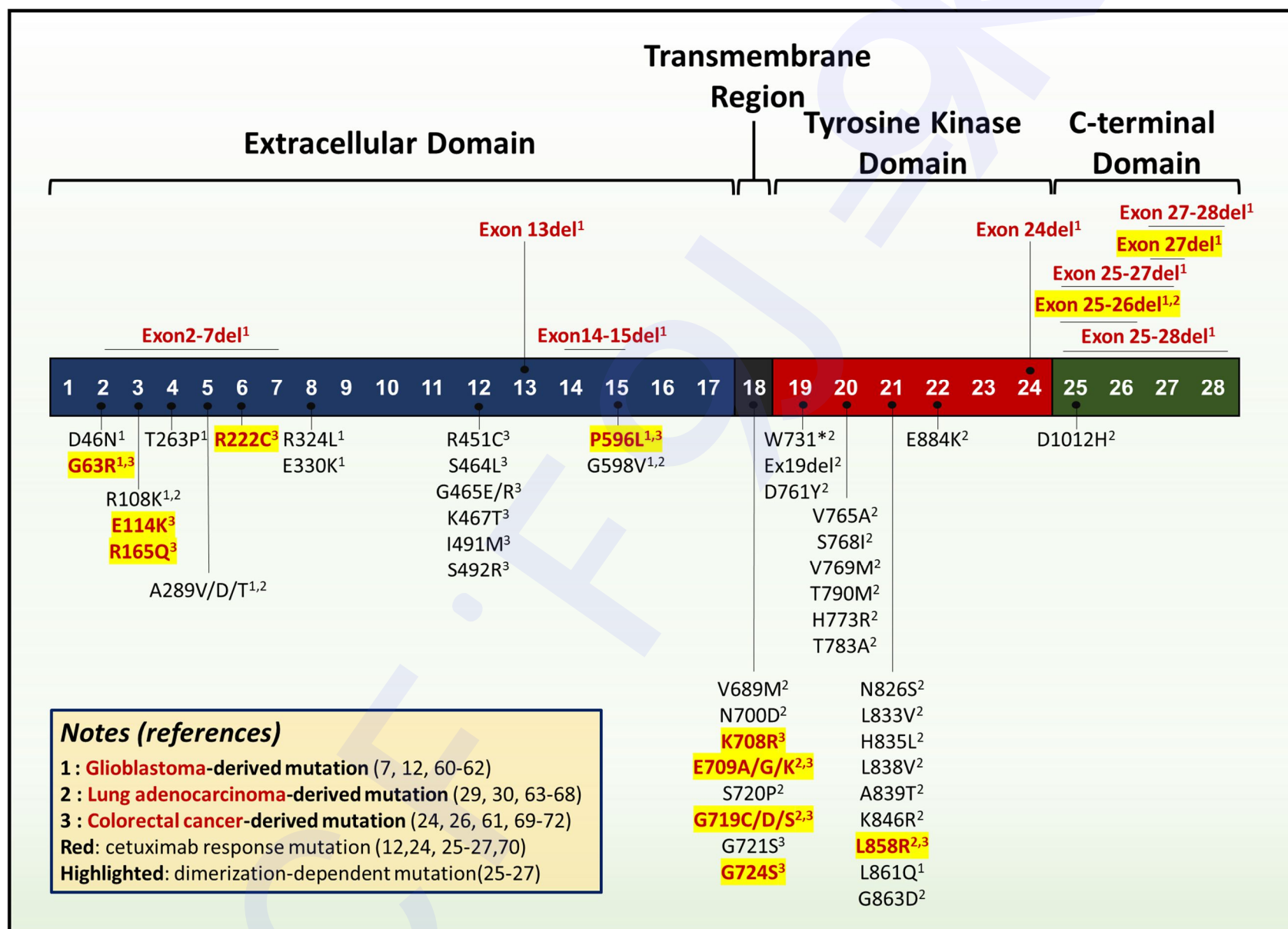


Figure 2. A subset of major *EGFR* mutations derived from cancer-patients are presented according to the location of each exon. Confirmed dimerization-dependent mutations and cetuximab-sensitive mutations are specifically indicated in the schematic diagram as shown in notes.

Mig6-mediated negative feedback mechanisms regulating EGFR activity

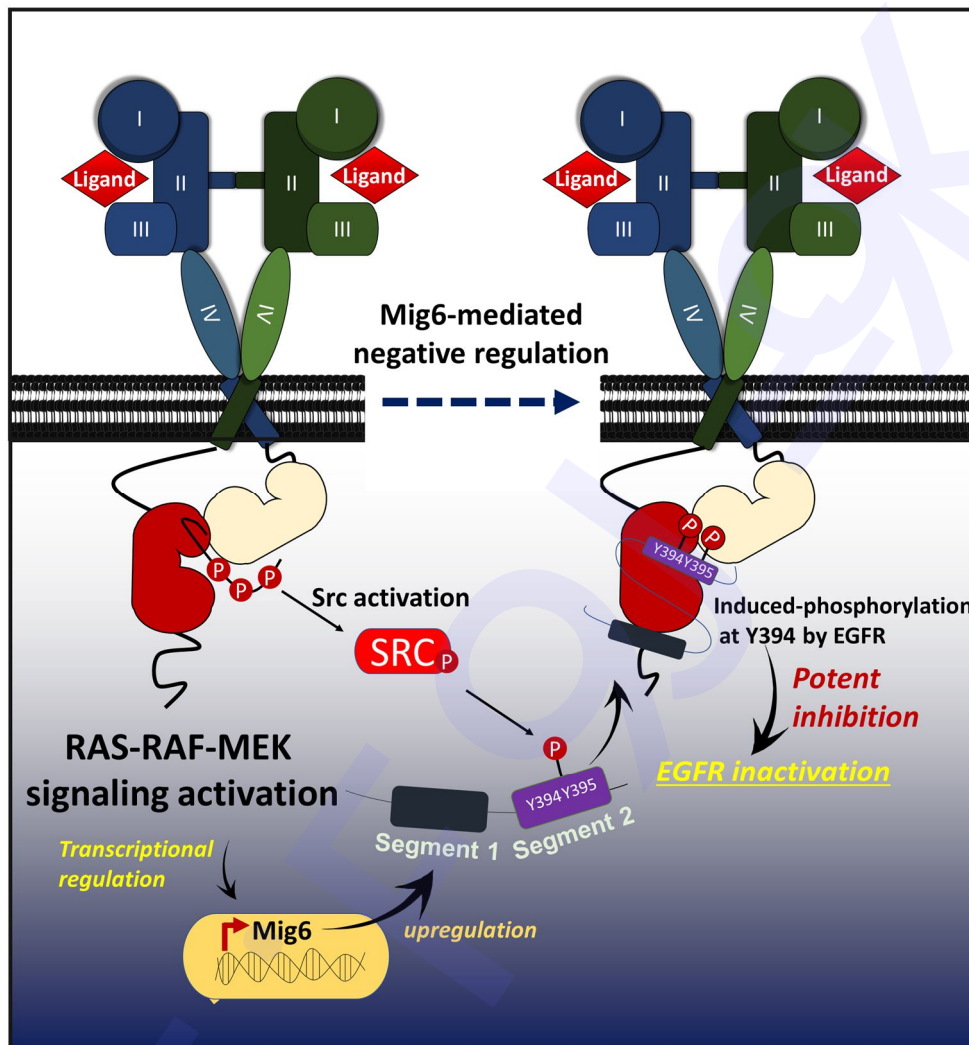


Figure 3. Model for mechanism of feedback inhibition of EGFR by Mig6 (adapted from Park et al., 2015).

Activated EGFR upregulates the expression of Mig6 via the Ras-RAF-Map kinase signaling pathway. Mig6 segment 1 binds to dimerization interface of EGFR monomer and block the further asymmetric dimerization. In addition, Src activated by EGFR phosphorylates Mig6 on Y395 in segment 2. This phospho-segment 2 of Mig6 interact with EGFR and is further phosphorylated on Y394, which renders it a potent inhibitor of EGFR. After phosphorylation, segment 2 bound to EGFR rearranges to block the peptide-substrate binding cleft. A single Mig6 protein should be sufficient to inactivate the wildtype receptor, as only one subunit in the asymmetric dimer is active and able to phosphorylate Mig6.