Mechanistic Insights into the Activation of Oncogenic Forms of EGF Receptor

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Abstract

Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that is commonly activated by mutation in non-small cell lung cancer. The mechanism of this oncogenic activation is incompletely understood but, in contrast to the WT EGFR, is proposed to be independent of kinase domain dimerization. Mechanistic studies on EGFR have largely relied on cell-based assays or isolated kinase domain measurements. Here we show using purified, near full-length EGFR proteins (tEGFRs) that two oncogenic mutants are fully active in the absence of EGF and highly resistant to the known therapeutic and endogenous inhibitors, Cetuximab, lapatinib, and MIG6. Based on the pattern of inhibition and the effects of additional asymmetric kinase dimer interface mutations, we propose that these oncogenic EGFR mutants drive and strongly depend on the formation of the asymmetric kinase dimer for activation, which has implications for drug design and cancer treatment strategies.

The epidermal growth factor receptor (EGFR) family comprises of four members in humans, EGFR (HER1), HER2/Neu, HER3, and HER4[1]. These receptor tyrosine kinases (RTKs), play consequential roles in a variety of solid cancers and are the targets of many successful antineoplastic therapeutics[2,3]. The synthetic compound erlotinib targets the active conformation of the kinase domain and is clinically approved for non-small cell lung cancer. Erlotinib is particularly effective in cancers in which the EGFR kinase domain contains activating mutations, the two most common of which are 746–750 and L858R[4–7]. The synthetic compound lapatinib is FDA-approved for the treatment of HER2/Neu-positive breast cancer and is proposed to bind preferentially to the inactive conformations of EGFR and Her2/neu[8,9] kinase domains. Cetuximab is an antibody that binds to the EGFR

ectodomain, blocking the binding of EGF to the receptor, and is approved for treatment of several EGFR-positive cancers [10,11].

EGFR family members are composed of a ligand-binding extracellular region, a membrane spanning region, a juxtamembrane region, a kinase domain, and a C-tail that can be autophosphorylated[12,13](Fig. 1a). Activation of EGFR by EGF involves the formation of a specific dimer of the extracellular ligand-binding regions[14–18], which appears to promote an asymmetric dimer interaction between the kinase domains in which the activity of one kinase subunit (acceptor kinase) is stimulated by another (donor kinase)[19]. The interface of this asymmetric dimer has been defined crystallographically and by mutagenesis and involves the N-terminal lobe (including Ile706) of the acceptor kinase and the C-terminal lobe (including Val948) of the donor kinase[19]. A peptide segment (segment 1) of the tumor suppressor protein MIG6 (RALT) has been shown to be a moderately potent inhibitor of EGFR kinase activity by binding to the C-lobe of the EGFR kinase domain and sterically blocking asymmetric dimer formation[20] (Fig. 1b). Another MIG6 segment C-terminal to segment 1 (segment 2) enhances the inhibitory activity of MIG6 and is believed to interact directly with the EGFR kinase active site[20].

Previous *in vitro* studies of the isolated L858R EGFR kinase domain have shown that it is ~50-fold more active relative to the WT kinase domain but does not appear to depend on asymmetric dimer formation[19,21]. The L858R EGFR kinase domain is, however, sensitive to erlotinib and MIG6 inhibition[20,22]. Tyrosine phosphorylation of MIG6 appears to be increased in cancer cell lines containing 746–750 or L858R EGFRs, suggesting that in addition to inhibiting EGFR, MIG6 may also be a direct substrate of these mutant receptor EGFRs[23]. There has been limited enzymologic characterization of the 746–750 EGFR kinase domain[24]. Cell-based assays with full-length L858R and 746–750 EGFRs show enhanced autophosphorylation of the EGFR C-terminal tails and other proteins relative to WT EGFR[22,25,26], but the enzymologic basis for this increased phosphorylation has been difficult to establish because of the complex environment of the cell.

Previously, we demonstrated the feasibility of expressing, purifying, and analyzing the kinetics for near-full length EGFR (tEGFR, aa25–1022), which lacks only part of the C-terminal tail[27]. It was shown that the EGF bound form of WT tEGFR had a k_{cat} that was 150-fold greater and a sensitivity to erlotinib that was ~25-fold higher than the Cetuximab–bound form, consistent with EGF–driven activation[27]. Interestingly, and not readily understood based on the kinase crystal structures, the potencies of lapatinib for EGF– and Cetuximab–bound tEGFRs were found to be within 2-fold of one another[27].

To investigate the effects of cancer-associated EGFR mutations on the mechanism and degree of EGFR activation, we examine here the kinase activity of wild-type and mutant EGFRs in the presence and absence of inhibitors targeting different activation states of EGFR. Unexpectedly, we find that EGF-independent activation of L858R and 746–750 tEGFRs remains strongly dependent on asymmetric kinase dimer formation.

RESULTS

Expression and purification of L858R and 746–750 tEGFRs

The cDNAs encoding human EGFR aa 25–1022 (tEGFR) with mutations L858R or 746–750 were transiently transfected into 2–4 L of HEK 293 GnTi⁻ cells[28], and the mutant tEGFRs were purified from detergent-solubilized cell extracts as previously described using the anti-EGFR Ab 528[27,29]. Expression levels of the mutant tEGFRs were reduced compared to WT tEGFR (Fig. 1c), leading us to suspect that the oncogenic forms of tEGFR may be cytotoxic at high expression levels owing to excessive kinase activity[30,31] or unstable because of the mutation. We thus evaluated expression in the presence of the reversible EGFR inhibitor erlotinib, which indeed led to enhanced expression of both mutant tEGFRs at the relatively low inhibitor concentration of 50 nM (Fig. 1c). Addition of erlotinib to the cell culture media allowed for the purification of ~0.1 mg of L858R and 746–750 tEGFRs per liter of cell culture. An EGF–bound and a Cetuximab Fab–bound form of each tEGFR mutant were obtained by eluting the affinity column with either EGF or the Cetuximab Fab. These tEGFR complexes were further subjected to size exclusion chromatography, with the majority of tEGFRs eluting at positions consistent with formation of high molecular weight but self-limited oligomers. We estimated tEGFR purities to be >80% by SDS-PAGE (Fig. 1d). Extensive dialysis and chromatography were used to remove erlotinib prior to storage. Similar ATP K_m^{app} and k_{cat} values were observed from two different protein batches purified from cells cultured with either a high (2 μM) or low (50 nM, considerably below the tEGFR IC_{50}, vide infra) concentration of erlotinib, indicating that erlotinib was effectively removed during purification.

Kinetic parameters of the mutant tEGFRs

The mutant tEGFRs were assayed using a direct, radiometric assay in which transfer of phosphate from γ-^{32}P-ATP to a biotinylated peptide was monitored after avidin pull-down of radiolabeled phosphopeptide. Both L858R and 746–750 tEGFRs in their EGF and Cetuximab–bound forms display linear kinase activity versus time as well as enzyme concentration (Supplementary Fig. 1), suggesting that these mutant receptors are stable in the conditions of our assay. It is readily apparent from these results that the Cetuximab–bound forms of oncogenic mutant tEGFRs are dramatically more active than WT enzyme in the presence of Cetuximab, which shows poorly detectable kinase activity under similar conditions. The k_{cat} and K_m^{app} values for peptide and ATP substrates for WT and mutant tEGFRs are shown in Table 1 (Steady-state curves are shown in Supplementary Fig. 2). Striking differences in the k_{cat} values between the oncogenic mutant tEGFRs and WT tEGFR in the Cetuximab–bound complexes are observed. The k_{cat}s for Cetuximab–bound oncogenic tEGFRs are within 2-fold of each other but approximately 200-fold greater than the k_{cat} for Cetuximab–bound WT tEGFR. In contrast, the k_{cat}s for the EGF–bound WT and mutant tEGFRs are similar to each other and to the Cetuximab–bound forms of L858R and 746–750 tEGFRs. These results indicate that the activities of oncogenic tEGFRs are comparable or greater than that of EGF-stimulated WT tEGFR.

The ATP K_ms also show marked differences between mutant and WT tEGFRs. Compared to that of the WT EGF–tEGFR complex, the ATP K_ms of the L858R and 746–750 tEGFRs

are 50- to 200-fold higher. The finding of the elevated ATP K_m with L858R tEGFR is consistent with previously reported analysis of the isolated L858R kinase domain[21,24]. Since both Leu858 and the aa746–750 loop are distant from the ATP binding site and from each other, the origin of the elevated ATP K_ms is not readily apparent from X-ray crystal structures. In contrast to the widely divergent ATP K_ms in WT versus mutant tEGFRs, effects on peptide substrate K_ms from the mutations are less dramatic. However, the L858R mutant shows 3–4-fold higher peptide K_m value versus WT and 746–750 tEGFRs, which may reflect substrate binding interactions involving the activation loop on which L858 resides[19,32].

Inhibition of oncogenic tEGFRs by small molecule kinase inhibitors

We next examined ability of the ATP mimic AMP-PNP, as well as the therapeutic agents erlotinib and lapatinib, to inhibit oncogenic tEGFRs. AMP-PNP showed similar IC_{50}s for WT and mutant tEGFRs in the range of 80–200 μM (Supplementary Fig. 3). Taking into account the ATP concentration employed (10 μM) and the respective tEGFR ATP K_m^{app}s, the K_is of AMP-PNP are deduced to be ~5- to 8-fold higher for L858R (107 ± 19 μM) and 746–750 (160 ± 59 μM) compared with WT (21 ± 5 μM). While clearly an increase, this 5–8-fold change is substantially lower than the 50- to 200-fold K_m differences for ATP among these EGFR forms, suggesting that the ATP K_m differences result largely from changes in rate-determining steps in catalysis, rather than simply changes in binding affinities for nucleotide.

The apparent K_is for erlotinib and lapatinib were also measured for each tEGFR mutant (Table 2, Supplementary Fig. 4). The L858R and 746–750 tEGFR forms were potently inhibited by erlotinib with apparent K_is in the range of 0.1–0.4 μM, about equal to the apparent K_i of erlotinib for WT tEGFR complexed with EGF. However, compared with WT tEGFR, the oncogenic mutant tEGFRs showed similar potencies regardless of EGF or Cetuximab ligand. In contrast to the behavior of erlotinib, the L858R and 746–750 tEGFR forms were highly resistant to lapatinib with apparent K_is in the range of 2–6 μM. Taken together, these results suggest a model in which the L858R and 746–750 mutations drive tEGFR into an active conformation with diminished access to the inactive state, even relative to EGF–bound WT tEGFR.

MIG6 and tEGFR

The tumor suppressor protein MIG6 contains a 77 aa region composed of two functional regions, termed segments 1 and 2, that potently inhibits EGFR kinase activity in part by binding the C-lobe of the EGFR kinase domain and blocking asymmetric dimer formation[20]. Recent cellular proteomic studies have suggested that this region of MIG6 is also tyrosine phosphorylated in response to EGF[23]. We find that tEGFR efficiently phosphorylates MIG6 segment 1+2 on Tyr residues (Fig. 2a–b) and that both L858R and 746–750 tEGFRs catalyze the reaction ~10-fold better than WT tEGFR (Table 3, Supplementary Figs. 5). Interestingly, the level of autophosphorylation of WT tEGFR in reactions that contained MIG6 segment 1+2 was substantially inhibited relative to the oncogenic tEGFR forms (Fig. 2a). To more precisely measure the effects of MIG6 inhibition, we investigated the effects of both segment 1 (aa 334–364) and the entire 77 aa region (segment 1+2, aa 336–412) of

MIG6 on phosphorylation of our biotinylated EGFR substrate peptide, which could be readily separated from MIG6 using streptavidin-containing resin. In prior binding and inhibitory studies on the EGFR catalytic domain, segment 1 of MIG6 was sufficient to bind the C-lobe surface of the kinase domain but bound with ~100-fold weaker affinity relative to segment 1+2[20]. Segment 1 of MIG6 is a moderate inhibitor of EGF–bound WT tEGFR (IC_{50} = 100 μM) whereas L858R and   746–750 tEGFR are essentially resistant to inhibition by segment 1 (Supplementary Fig. 6). Segment 1+2 of MIG6 was a considerably more potent inhibitor of EGF–bound WT tEGFR with an IC_{50} of 0.5 μM, similar to its potency versus the isolated EGFR kinase domain (Fig. 2c) and the previously determined value versus the L858R EGFR kinase domain[20]. However, MIG6 segment 1+2 showed an IC_{50} of ~20 μM versus both L858R tEGFR and   746–750 tEGFR (Fig. 2c). Since the IC_{50} of MIG6 segment 1+2 is 0.4 μM for the isolated kinase domain of L858R[20], the far weaker inhibition of L858R tEGFR by MIG6 segment 1+2 suggests that the C-lobe of the kinase domain in the near full-length mutant protein is much less accessible to MIG6 interaction.

Role of Asymmetric Dimer in Oncogenic tEGFRs

Mutations in the N-lobe (I706Q) or C-lobe (V948R) region of the asymmetric kinase dimer interface have been shown to impair EGF induced activation of WT EGFR in cells[19,20]. However, the double mutant L858R V948R in the isolated EGFR kinase domain has been shown to have essentially identical catalytic activity to the isolated L858R EGFR kinase domain suggesting that dimerization is not important for its constitutive activation[20]. To investigate this issue in tEGFR, we introduced simultaneous L858R and I706Q substitutions into tEGFR and measured their effects on catalytic activity in the presence of EGF (Fig. 3a–b, Supplementary Fig. 2b). In the presence of EGF, this double mutant tEGFR showed a kinase rate that was ~30-fold lower than the single mutant L858R tEGFR, but showed a similar ATP K_m (Table 1, Supplementary Fig. 2b). Interestingly, in the presence of Cetuximab, the kinase rate of L858R/I706Q tEGFR was about 10-fold lower than the EGF–bound form, close to the limit of detection (Fig. 3a–b). These results suggest that the kinase domain asymmetric dimer interface in L858R tEGFR contributes substantially to its constitutive kinase activity and that EGF can still promote dimerization in the presence of an N-lobe interface mutation.

To exclude the possibility that I706Q was affecting tEGFR kinase activity independent of dimer interface effects, we prepared and analyzed L858R/V948R tEGFR. As shown in Figure 3a–b, the kinase activities of L858R/V948R tEGFR in its EGF– and Cetuximab– bound forms were reduced substantially compared to that of L858R tEGFR, indicating that mutation of either the N-lobe or C-lobe face of the dimer interface of L858R results in a 10- to 30-fold decrease in catalytic rate. To probe the conformational properties of L858R/ I706Q tEGFR further, we examined its sensitivity to inhibition by lapatinib (Supplementary Fig. 4b). As shown in Table 2, the apparent K_i of lapatinib for EGF–bound L858R I706Q tEGFR is 700 nM, about 6-fold lower than the apparent K_i of lapatinib for EGF–bound L858R tEGFR. These observations indicate that activation by the L858R substitution is coupled to kinase dimer formation and that inhibiting dimer formation by mutation increases accessibility of the inactive kinase conformation.

Next, we investigated the kinase activity of (746–750) I706Q tEGFR (Fig. 3c). In the EGF–bound form, (746–750) I706Q tEGFR showed a kinase rate that was 5-fold lower than that of 746–750 tEGFR. There was a further 3-fold reduction in kinase activity with the Cetuximab–bound form of (746–750) I706Q tEGFR relative to the EGF–bound form. Taken together, these data suggest that the asymmetric dimer interface also remains important for activation of tEGFR with the aa746–750 loop deletion, although it appears that there is a larger amount of residual kinase activation in (746–750) I706Q tEGFR relative to L858R I706Q tEGFR.

DISCUSSION

This study finds that the two most common EGFR mutations in non-small cell lung cancer (L858R and 746–750) result in EGF–independent tEGFR activities comparable to, or greater than, the activity of EGF–stimulated WT EGFR, and that tEGFR activation resulting from these mutations remains strongly coupled to asymmetric kinase dimer formation (Fig. 3d). This model differs from prior enzymatic studies on the isolated L858R EGFR kinase domain[20] that suggested the asymmetric dimer interaction is dispensable for stimulation of catalysis. In contrast to the isolated L858R kinase domain results, we observe that mutation of residues in the dimer interface in tEGFR dramatically reduces the activity of both the L858R and 746–750 tEGFRs, indicating that this dimerization event is critical for activation of both of these oncogenic mutants.

Strong resistance to MIG6 inhibition further highlights the loss of accessibility of the asymmetric kinase dimer interface in the purified oncogenic EGFRs, as activation appears to both remain coupled to and drive kinase dimer formation. Size exclusion chromatography suggests that L858R and 746–750 tEGFR proteins are oligomeric, presumably reflecting chains of tEGFR formed through a series of asymmetric kinase interactions. However, the fact that these mutants do not shift completely to the column void volume suggests that the chains are self-limiting even in the absence of membrane. The thermodynamic inter-dependency of the active kinase conformation and dimerization provides a plausible model for these results (Fig. 3d). The previous report that activation is dimer-independent in the isolated EGFR kinase domain underscores the limitations of attempting to understand the regulation of this complex receptor by studying isolated fragments. The multiple domains of full length EGFR clearly contribute to a web of intra- and inter-molecular interactions, including the juxtamembrane region[13,33–35], that couple the effects of activating mutations and dimer formation and are central to the regulation mechanism. The lack of a membrane bilayer and the truncation of the C-tail in our tEGFR studies may affect the results, but the fact that we recapitulate a high degree of kinase activation with the wild type EGFR enzyme in its EGF– vs. Cetuximab–bound forms increases confidence in the relevance of our findings with the mutant proteins.

The observation that L858R EGFR transfected in cells retains EGF dependency[20,23] differs from our findings with purified tEGFR. This difference may result from the indirect measurement of kinase activity in cells, EGF effects on cellular trafficking/internalization, or the presence of inhibitory feedback mechanisms that EGF is able to override in cells. In any event, we believe that our data indicating loss of EGF–dependence for L858R reflect the

intrinsic properties of the purified oncogenic mutant EGFRs and set a benchmark for analyzing cellular behavior of these molecules. The presence of heterodimers of oncogenic forms of EGFR with WT EGFR or HER2–4 may also affect the ligand-dependency of oncogenic EGFR variants in a cellular context.

The studies reported here also enhance our understanding of the paradoxical inhibitory properties of lapatinib with WT tEGFR in its EGF– and Cetuximab–bound forms[27]. The unexplained parity in potency of lapatinib between the EGF– and Cetuximab–bound WT tEGFR forms was initially interpreted as potentially due to lapatinib binding favorably in distinct modes to active as well as inactive kinase conformations. Based on the >20-fold reduced potencies of lapatinib for the L858R and 746–750 tEGFRs compared to EGF–bound WT tEGFR, we hypothesize now that the kinase active conformation of EGF–bound WT EGFR may be nearly isoenergetic with the kinase inactive state of EGF–bound WT EGFR and largely present in the donor kinase. In this way, there would be little thermodynamic impediment to lapatinib accessing the kinase inactive conformation of EGF–bound WT EGFR. Consistent with this model, we have found that MIG6 segment 1+2 potently inhibits EGF–bound WT tEGFR, presumably because this asymmetric dimer is not highly stabilized, allowing access of the C-lobe of the kinase domain to efficiently bind segment 1 of MIG6. In contrast, MIG6 segment 1+2 cannot effectively inhibit the oncogenic mutant tEGFRs, presumably because the C-lobe is less accessible in conditions more strongly favoring formation of asymmetric kinase dimers.

We also show here that MIG6 is tyrosine phosphorylated by tEGFR, particularly the L858R and 746–750 forms. These results extend a prior study showing how specific increases in these substrates in isogenic, mutant EGFR tumor cell lines resulted in enhanced tyrosine-phosphorylation of MIG6[23]. How tyrosine phosphorylation of MIG6 might impact its interaction with EGFR or have other influences in signaling is an important direction for future studies. It is also interesting that the apparent K_m of L858R tEGFR for substrate peptide is ~4-fold greater than for WT and 746–750 tEGFRs. This difference implies that oncogenic EGFR mutations may affect the specificity or extent of substrate phosphorylation, which will be an interesting question to address with future proteomics studies[36–38].

Based on the findings here, the dramatic increase in ATP K_m for L858R and 746–750 tEGFR forms can be understood to be partially related to altered nucleotide affinities but also likely to result from changes in microscopic rate constants for the enzymatic transformation. For example, if the chemical (phosphoryl transfer) step in the mutant kinases is greatly accelerated relative to the WT reaction, this rate increase might result in an elevated ATP K_m[39]. From a functional perspective, however, ATP K_ms in the 200–700 μM range are of little consequence in the cell since ATP concentrations are in the millimolar range.

These studies have several implications for cancer therapeutic strategies. Given their resistance to lapatinib and Cetuximab, there appears to be little rationale for the use of these agents in non-small cell lung cancer containing L858R and 746–750 EGFR mutations whereas erlotinib's clinical benefit in these cases is readily understood. Furthermore, targeting cancers with these oncogenic mutations with MIG6 peptidomimetics appears to be

a challenging proposition since competition for this dimer interface will be difficult. In future experiments with tEGFRs, it will be interesting to determine the effects of the gatekeeper T790M drug resistance mutation[40] on the relative activation and inhibitor sensitivities of these enzymes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

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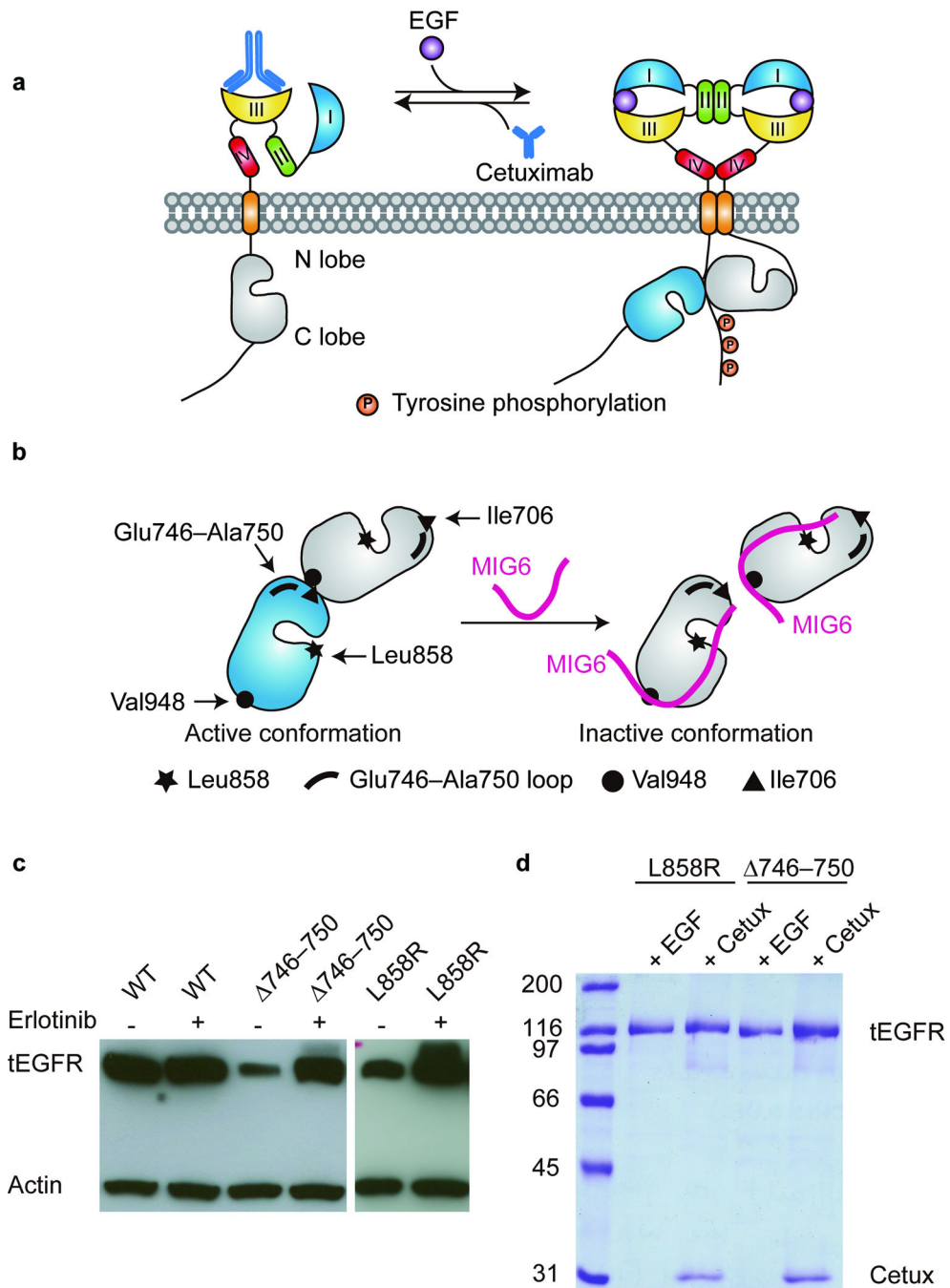
Figure 1. Activation and inhibition mechanism for WT EGFR and the expression and purification strategy for mutant tEGFRs

(a) Unliganded and Cetuximab–bound WT EGFR exist primarily in the tethered conformation. EGF binding to the ectodomain initiates formation of specific receptor-mediated dimers and activation of the intracellular kinase domain via formation of an asymmetric dimer. The active conformation of kinase domain is depicted as blue and the inactive conformation is depicted as gray. Cetuximab is shown in light blue and EGF is shown in purple. Not to scale. (b) MIG6 inhibits WT EGFR by binding to the C-lobe of the EGFR kinase domain and blocking the asymmetric dimer interface. Sites of key residues

studied here are highlighted. (c) Western blot analysis of the expression levels of WT, L858R, and Δ746–750 tEGFRs in the presence and absence of the EGFR inhibitor erlotinib. HEK293 GnTi⁻ cells were transfected with the plasmid DNA encoding tEGFR, and cultured in the presence and absence of 50 nM erlotinib. (d) Coomassie blue-stained SDS-PAGE analysis of the purified L858R tEGFR and Δ746–750 tEGFR with either EGF or Cetuximab (Cetux) as ligand.
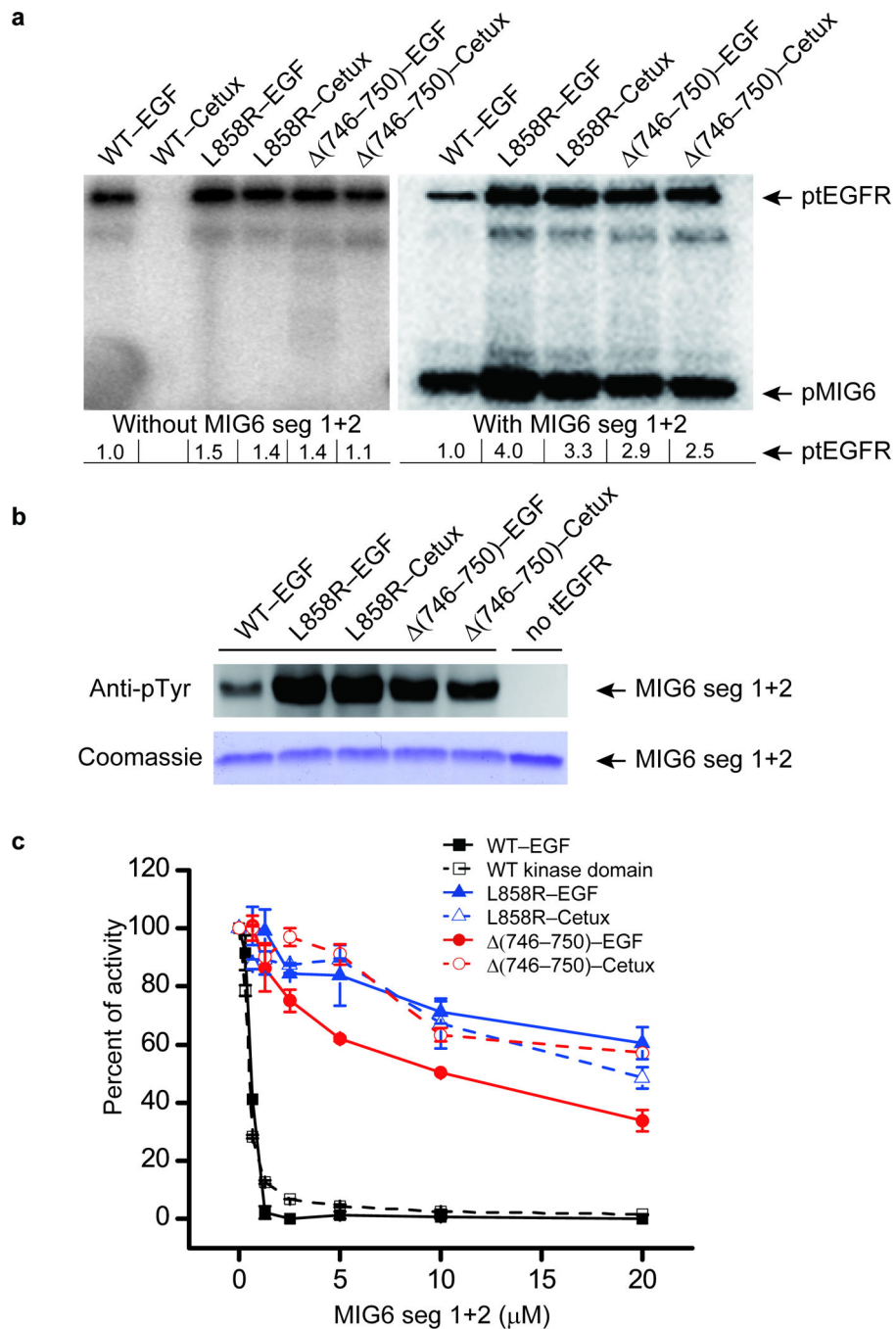
Figure 2. MIG6 seg 1+2 interacts with WT and mutant tEGFRs

(a) Autoradiograph showing incorporation of radioactive phosphate (^{32}P) into tEGFR and MIG6 simultaneously by *in vitro* phosphorylation of MIG6 seg 1+2 (77 aa) using various tEGFR forms. MIG6 seg 1+2 was incubated with [^{32}P] ATP and WT–EGF, L858R–EGF, L858R–Cetux, (746–750)–EGF, and (746–750)–Cetux tEGFRs. Left, negative control in which no MIG6 seg 1+2 was added to the reaction buffer. The relative intensity of each tEGFR band (divided by the intensity of the EGFR band on WT–EGF lane from each autoradiograph) is shown below. (b) MIG6 seg 1+2 is phosphorylated on tyrosine by

tEGFRs *in vitro*. MIG6 seg 1+2 was incubated with ATP in the presence and absence of tEGFRs (WT tEGFR–EGF, L858R tEGFR–EGF, L858R tEGFR–Cetux,   (746–750) tEGFR–EGF, and   (746–750) tEGFR–Cetux). The tyrosine phosphorylation of MIG6 seg 1+2 was probed with anti-pY 4G10 antibody (upper panel). The coomassie staining of MIG6 seg 1+2 (lower panel) indicates that the total amount of MIG6 from each lane was identical. (c) The inhibition effects of MIG6 seg 1+2 on WT–EGF, WT kinase domain (residues 668–1210), L858R–EGF, L858R–Cetux,   (746–750)–EGF, and   (746–750)–Cetux tEGFRs. Percent of activity values (± s.d.) calculated from three independent experiments are shown.
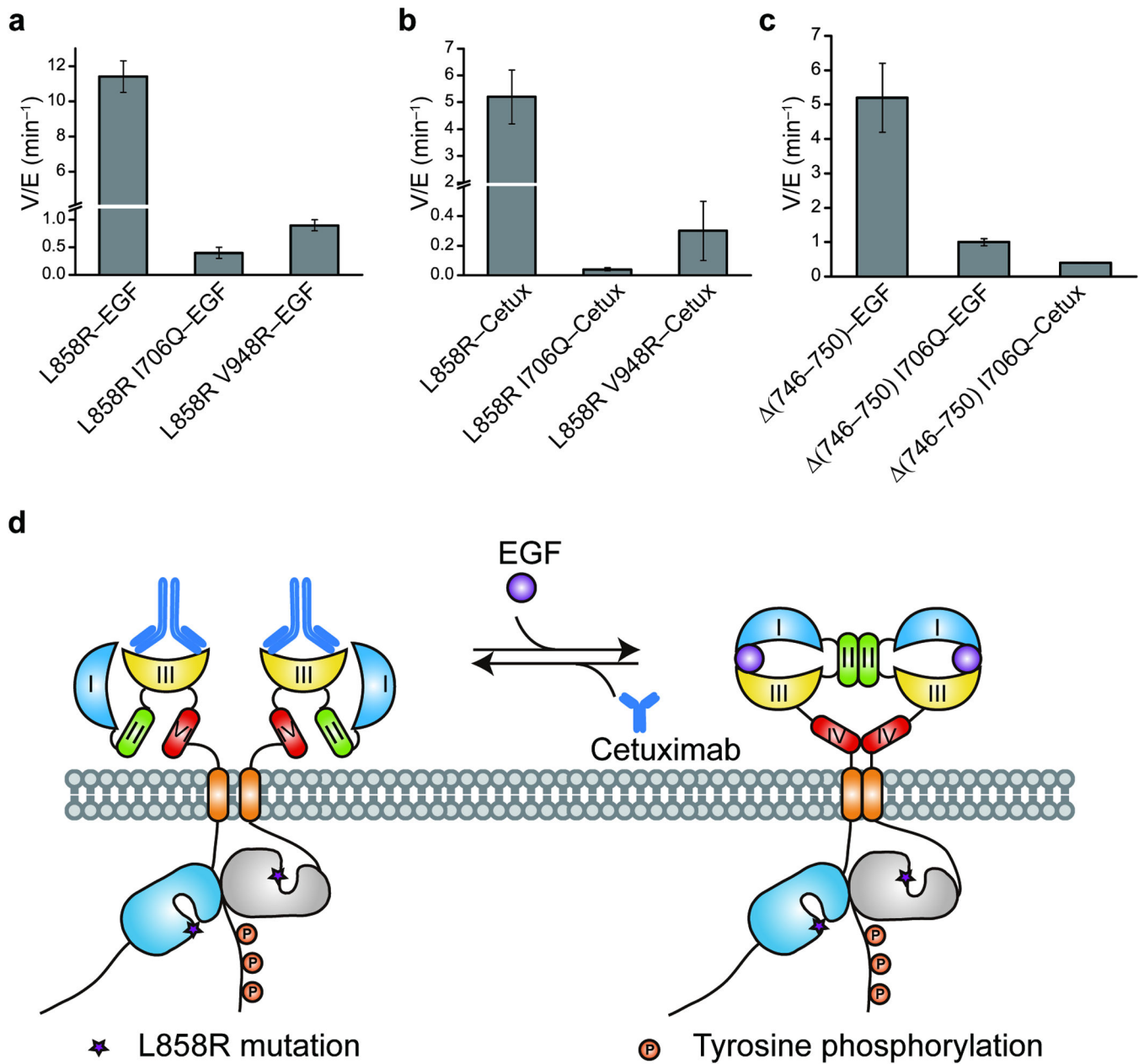
Figure 3. Role of the asymmetric dimer interface for oncogenic EGFR activation
(a) Comparison of the specific activities of L858R–EGF, L858R I706Q–EGF, and L858R V948R–EGF tEGFRs. (b) Comparison of the specific activities of L858R–Cetux, L858R I706Q–Cetux, and L858R V948R–Cetux tEGFRs. (c) Comparison of the specific activities of Δ(746–750)–EGF, Δ(746–750) I706Q–EGF, and Δ(746–750) I706Q–Cetux tEGFRs. (d) Activation mechanism for EGFR oncogenic mutations. The unliganded mutant EGFR is present in the super-activated dimer conformation which is driven by kinase domain mutation (L858R shown or Δ746–750, not shown) and independent of ectodomain occupancy.

Table 1

Enzymatic parameters of WT, 746–750, L858R, and L858R I706Q tEGFRs with EGF or Cetuximab as ligand.

Protein (tEGFR)	Ligand	K_m^{app} peptide (μM)	K_m^{app} ATP (μM)	k_{cat}^{app} (min^{-1})
WT[*27]	EGF	12 ± 2	3.6 ± 0.3	18 ± 1
WT[*27]	Cetuximab	23 ± 5	25 ± 4	0.13 ± 0.01
746–750	EGF	13 ± 1	670 ± 130	46 ± 9
746–750	Cetuximab	21 ± 4	640 ± 160	36 ± 5
L858R	EGF	45 ± 4	180 ± 30	21 ± 1
L858R	Cetuximab	64 ± 6	420 ± 140	17 ± 3
L858R I706Q	EGF	34 ± 7	138 ± 49	0.9 ± 0.1

[*] Indicates data was previously reported[27]. Enzymatic parameters (± s.d.) calculated from duplicated experiments are shown.

Table 2

Inhibitory effects of erlotinib and lapatinib on WT, 746–750, L858R, and L858R I706Q tEGFRs with EGF or Cetuximab as ligand.

Protein (tEGFR)	Ligand	K_i^{app} for erlotinib (μM)	K_i^{app} for lapatinib (μM)
WT[*27]	EGF	0.129 ± 0.024	0.07 ± 0.01
WT[*27]	Cetuximab	3.1 ± 1.5	0.04 ± 0.01
746–750	EGF	0.135 ± 0.003	1.86 ± 0.73
746–750	Cetuximab	0.423 ± 0.055	6.19 ± 0.95
L858R	EGF	0.100 ± 0.008	4.54 ± 0.59
L858R	Cetuximab	0.275 ± 0.011	3.48 ± 0.34
L858R I706Q	EGF	N.A.	0.70 ± 0.08

[*]Indicates data was previously reported[27]. N.A. stands for Not Assayed. K_i^{app} values (± s.d.) calculated from duplicated experiments are shown.

Table 3

Enzymatic parameters of MIG6 seg 1+2 phosphorylation by WT, 746–750, and L858R tEGFRs with EGF or Cetuximab as ligand.

Protein (tEGFR)	Ligand	K_m^{app} MIG6 (µM)	K_m^{app} ATP (µM)	k_{cat}^{app} (min^{-1})
WT	EGF	8 ± 2	10 ± 2	1.0 ± 0.1
746–750	EGF	13 ± 5	300 ± 140	8 ± 2
746–750	Cetuximab	4 ± 1	N.A.	N.A.
L858R	EGF	13 ± 3	34 ± 12	9 ± 1
L858R	Cetuximab	12 ± 2	120 ± 50	14 ± 3

N.A. stands for not assayed. Enzymatic parameters (± s.d.) calculated from duplicated experiments are shown.