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Targeting Epidermal Growth Factor Receptor for Cancer Treatment: Abolishing Both Kinase-Dependent and Kinase-Independent Functions of the Receptor

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Abstract——**Epidermal growth factor receptor (EGFR), a receptor tyrosine kinase, is activated by ligand binding, overexpression, or mutation. It is well known for its** **tyrosine kinase-dependent oncogenic activities in a variety of human cancers. A large number of EGFR inhibitors have been developed for cancer treatment, including**

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monoclonal antibodies, tyrosine kinase inhibitors, and a vaccine. The EGFR inhibitors are aimed at inhibiting the activation or the activity of EGFR tyrosine kinase. However, these agents have shown efficacy in only a few types of cancers. Drug resistance, both intrinsic and acquired, is common even in cancers where the inhibitors have shown efficacy. The drug resistance mechanism is complex and not fully known. The key vulnerability of cancer cells that are resistant to EGFR inhibitors has not been identified. Nevertheless, it has been increasingly recognized in recent years that EGFR also possesses kinase-independent oncogenic functions and that these noncanonical functions may play a crucial role in cancer resistance to EGFR inhibitors. In this review, both kinase-dependent and -independent activities of EGFR are discussed. Also discussed are the mechanisms of actions and therapeutic activities of clinically used EGFR inhibitors and sustained EGFR overexpression and EGFR interaction with other receptor tyrosine kinases to counter the EGFR inhibitors. Moreover, this review discusses emerging experimental therapeutics that have shown potential for overcoming the limitation of the current EGFR inhibitors in preclinical studies. The findings underscore the importance and feasibility of targeting both kinase-dependent and -independent functions of EGFR to enhance therapeutic efficacy and minimize drug resistance.

Significance Statement——EGFR is a major oncogenic driver and therapeutic target, but cancer resistance to current EGFR inhibitors remains a significant unmet clinical problem. This article reviews the cancer biology of EGFR as well as the mechanisms of actions and the therapeutic efficacies of current and emerging EGFR inhibitors. The findings could potentially lead to development of more effective treatments for EGFR-positive cancers.

I. Introduction

A. The Human Epidermal Growth Factor Receptor Family Receptor Tyrosine Kinases

Epidermal growth factor receptor (EGFR), also known as ErbB1 or HER1, which was discovered by Cohen and coworkers in 1978 (Carpenter et al., 1978), is a member of the human epidermal growth factor receptor (HER) family of four closely related receptor tyrosine kinases (RTKs). The other HER family members include HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). The HER receptors are single-pass transmembrane proteins, composed of an extracellular domain (ECD), a transmembrane domain, an intracellular tyrosine kinase domain, and a C-terminal tail. Upon ligand binding to the ECD of the RTKs or their own overexpression, the receptors undergo homo- or heterodimerization, which results in tyrosine kinase activation and auto- or trans-phosphorylation of tyrosine residues in the C-terminal tail. The phosphotyrosine sites recruit adaptor proteins, signaling proteins, and regulatory proteins to activate various growth signaling pathways, such as the RAS/RAF/MEK/ERK pathway and the PI3K/AKT/mTOR pathway (Fig. 1) (Wee and Wang, 2017). However, HER3 kinase is almost totally inactive due to nonconservative substitution of several amino acids in its kinase domain, but exerts potent oncogenic activity by relying on transphosphorylation by another RTK with which it heterodimerizes (Beji et al., 2012; Lee et al., 2009; Lyu et al., 2018). EGFR can also function without requiring its kinase activity, as described later. The HER RTKs play important roles in various developmental and physiologic processes, but excessive activity resulting from overexpression or activating mutation drives cancer development and progression (Sibilia et al., 2007; Roskoski, 2014).

B. Epidermal Growth Factor Receptor Overexpression and Mutation in Cancer

GFR is overexpressed in a variety of human cancers. High tumor EGFR expression is linked to poor prognosis in bladder cancer (Neal et al., 1990), breast cancer (Lee et al., 2015b), cervical cancer (Tian et al., 2016), esophageal cancer (Jiang et al., 2015), head and neck cancer (Chung et al., 2011), ovarian cancer (Psyrri et al., 2005), and stomach cancer (Galizia et al., 2007). EGFR is also overexpressed in colorectal cancer (CRC), non-small cell lung cancer (NSCLC), and glioblastoma multiforme (GBM), but the prognostic significance of EGFR overexpression is not observed in NSCLC and GBM (Hirsch et al., 2003; Heimberger et al., 2005) and is controversial in CRC (Spano et al., 2005; Rego et al., 2010; Hong et al., 2013). The mechanism of EGFR overexpression is not fully understood. EGFR overexpression results mainly from gene amplification in GBM (Viana-Pereira et al., 2008), but in CRC and NSCLC, *EGFR* is amplified in about 10% to 16% of CRC cases and about 10% of NSCLC cases, while EGFR is overexpressed in about 60% of the cases in both diseases (Hirsch et al., 2003; Shia et al., 2005; Spano et al., 2005; Kato et al., 2019). Gene amplification is not the main driver of EGFR overexpression in head and neck squamous cell carcinoma (HNSCC) as well (Bei et al., 2004; Maiti et al., 2013). Several studies show that EGFR expression may be induced by its own ligand through increasing protein synthesis (Clark et al., 1985; Kudlow
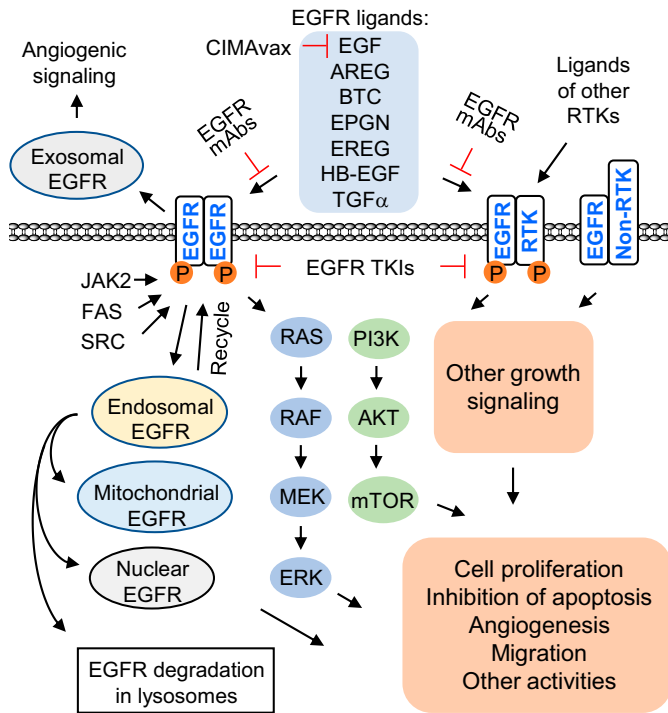
Fig. 1. EGFR signaling and trafficking and the effects of EGFR inhibitors. EGFR is activated by seven ligands. EGFR may also be activated by overexpression; by heterodimerizing with other RTKs; or by Janus tyrosine kinase 2, fatty acid synthase, and SRC. Other RTKs may be activated by binding to EGFR. EGFR may also bind to and stimulate the activity of non-RTK membrane proteins such as sodium/glucose cotransporter 1. Activation of EGFR and its binding partners initiate multiple growth signaling pathways, leading to cell proliferation and other changes. Activated EGFR may be internalized and delivered to lysosome for degradation; be recycled to cell membrane to maintain signaling; or exert growth signaling in endosome, mitochondria, nucleus, and exosome. EGFR heterodimerization may prevent EGFR internalization. EGFR mAbs and TKIs as well as CIMAvas only partially inhibit the EGFR signaling network.

et al., 1986; Yoshida et al., 1989). Activating EGFR mutation is common only in NSCLC and GBM. EGFR is mutated in its kinase domain in NSCLC in 10% to 20% of the cases in North America and Europe and 40% to 64% of the cases in Asia (Midha et al., 2015). In contrast, EGFR is activated by point or deletion mutations in its ECD in GBM (An et al., 2018). EGFRvIII, an in-frame deletion of exons 2 to 7 removing 267 amino acids, is the most common EGFR mutant in GBM and occurs in 20% to 30% of the cases (Heimberger et al., 2005; Gan et al., 2013).

C. Clinically Used Epidermal Growth Factor Receptor Inhibitors

Reflecting the critical importance of EGFR as a therapeutic target in cancer, a total of 20 direct EGFR inhibitors have been developed over the past two decades, which include tyrosine kinase inhibitors (TKIs) and monoclonal antibodies (mAbs) (Table 1). Since the approval of gefitinib as the first EGFR TKI in 2003, 15 additional EGFR TKIs have been developed. The EGFR TKIs are either reversible or irreversible inhibitors and are approved either in the United States (gefitinib,

erlotinib, lapatinib, afatinib, brigatinib, dacomitinib, mobocertinib, neratinib, osimertinib, pyrotinib, and vandetanib) or outside the United States (icotinib, almonertinib, simotinib, lazertinib, and olmutinib). The EGFR TKIs were recently reviewed (Abourehab et al., 2021), except for mobocertinib (Gonzalvez et al., 2021; Han et al., 2021a) and lazertinib (Dhillon, 2021; Heppner et al., 2022), both of which were approved in 2021. Some of the EGFR TKIs are also inhibitors of other RTKs. Lapatinib also inhibits HER2 and is known as a dual EGFR/HER2 TKI. Afatinib, dacomitinib, neratinib, and pyrotinib also inhibit HER2 and HER4 and are known as pan-HER TKIs. Brigatinib and vandetanib are multikinase inhibitors. Brigatinib also inhibits anaplstic lymphoma kinase, FMS-like tyrosine kinase 3, and ROS1. Vandetanib also inhibits vascular endothelial growth factor receptor-1/2/3 and RET. Since the approval of cetuximab as the first EGFR mAb in 2004, three additional EGFR mAbs have been developed. Three EGFR mAbs are approved in the United States, including cetuximab, panitumumab, and necitumumab, and another, nimotuzumab, is approved outside the United States (Ramakrishnan et al., 2009; Yao et al., 2018; Cai et al., 2020). In addition to the EGFR TKIs and mAbs, a vaccine known as CIMAvax was developed in Cuba and entered the first clinical trial in 1998 there (González et al., 1998). CIMAvax inhibits EGFR activation by generating antibodies against circulating epidermal growth factor (EGF), one of the EGFR ligands (Rodríguez et al., 2010).

D. Clinical Activities of Epidermal Growth Factor Receptor Inhibitors

Although EGFR is implicated as an oncogenic driver in a variety of human cancers as described before, the EGFR inhibitors have shown clinical efficacy only in a few types of cancers. The EGFR TKIs are approved only for treating NSCLC harboring activating EGFR mutation, except for lapatinib, neratinib, pyrotinib, and vandetanib. Lapatinib, neratinib, and pyrotinib, which also inhibit HER2, are approved for treating HER2-positive breast cancer (Table 1). Vandetanib, which is a multikinase inhibitor, is approved for treating medullary thyroid cancer (Table 1). The EGFR mAbs are approved for treating CRC, HNSCC, or squamous cell lung carcinoma (squamous NSCLC) depending on the mAb, except for nimotuzumab, which is approved outside the United States for treating HNSCC, glioma, and nasopharyngeal carcinoma (Table 1). CIMAvax has not been approved in the United States, but a phase 1 trial there showed that CIMAvax is safe in NSCLC patients (Evans et al., 2022). The US Food and Drug Administration

TABLE 1
Clinically used EGFR inhibitors

| Agent type	Agent	Effect on EGFR kinase	Cancer treatment	References
TKI	Brigatinib	Reversible inhibition of kinase	NSCLC	Abourehab et al., 2021; Dhillon, 2021; Gonzalvez et al., 2021; Han et al., 2021a
	Elotinib		NSCLC	
	Gefitinib		NSCLC	
	Icotinib*		NSCLC	
	Lapatinib		HER2-positive breast cancer	
	Simotinib*		NSCLC	
	Vandetanib		Medullary thyroid cancer	
	Afatinib	Irreversible inhibition of kinase	NSCLC	
	Almonertinib*		NSCLC	
	Dacomitinib		NSCLC	
	Lazertinib*		NSCLC	
	Mobocertinib		NSCLC	
	Neratinib		HER2-positive breast cancer	
	Olmutinib*		NSCLC	
	Osimertinib		NSCLC	
	Pyrotinib		HER2-positive breast cancer	
mAb	Cetuximab	Inhibition of ligand activation of kinase	CRC, HNSCC	Ramakrishnan et al., 2009; Yao et al., 2018; Cai et al., 2020
	Panitumumab		CRC	
	Necitumumab		NSCLC	
	Nimotuzumab*		HNSCC, glioma, nasopharyngeal carcinoma	

*These drugs are approved for clinical use outside the United States.

denied approval of poziotinib (an EGFR TKI) for patients with NSCLC in 2022 on the grounds that benefit does not outweigh risk (http://bit.ly/3Vy8TkB). Even in cancers where the drugs show clinical efficacy, intrinsic and acquired drug resistance is common, as discussed in Section IV. Cancer resistance to EGFR inhibitors and the complex mechanisms of resistance have been the subjects of numerous reviews, some of which are cited here (Bertotti and Sassi, 2015; Westover et al., 2018; Leonetti et al., 2019; Parseghian et al., 2019; Pan and Magge, 2020). However, translating the knowledge of drug resistance mechanisms to benefit patients has been very challenging. For example, activating KRAS mutation was found to confer resistance to EGFR inhibitors in NSCLC, and KRAS^{G12C} is the most common activating KRAS mutant in NSCLC (Kempf et al., 2016). However, in a phase 3 clinical trial, sotorasib, which inhibits KRAS^{G12C}, achieved median progression-free survival at 5.6 months in NSCLC patients whose tumors harbor this mutation, compared with docetaxel, which targets microtubule and achieved median progression-free survival at 4.5 months (de Langen et al., 2023). KRAS mutation also renders CRC resistant to EGFR mAbs, but sotorasib achieves objective response rate of only 7% to 10% in patients whose tumors harbor KRAS^{G12C} (Hong et al., 2020; Fakih et al., 2022). Even in patients whose tumors respond to sotorasib, response is not durable. The key vulnerability of therapy-resistant cancer cells remains unknown. The EGFR inhibitors inhibit the activation or activity of EGFR tyrosine kinase but not its kinase-independent oncogenic functions.

II. Kinase-Dependent Activities of Epidermal Growth Factor Receptor

A. Ligand-Dependent and -Independent Activation of Epidermal Growth Factor Receptor Kinase

EGFR and its family members are best known for its kinase-dependent signaling from cell surface membrane. EGFR is stimulated by seven ligands with varying affinity (Olayioye et al., 2000; Singh et al., 2016). Four ligands are specific to EGFR, including EGF, transforming growth factor α (TGFα), amphiregulin (AREG), and epigen. Three ligands bind to both EGFR and HER4, including heparin-binding EGF-like growth factor, epiregulin, and betacellulin. Ligand binding to the ECD of EGFR induces homo- and heterodimerization, activation of tyrosine kinase, and phosphorylation of tyrosine residues in the C-terminal tail. These phosphotyrosine sites act as docking locations for a variety of proteins, which trigger cascades of downstream growth signaling (Fig. 1). In-depth molecular details of the activation of EGFR tyrosine kinase may be found in a recent review (Wee and Wang, 2017). EGFR may also form inactive dimer before ligand binding and kinase activation (Yu et al., 2002; Hajdu et al., 2020). In fact, all HER family receptors may pre-form homo- and heterodimers on the cell surface (Tao and Maruyama, 2008). Cancer cells may also overexpress EGFR ligands to enhance its oncogenic signaling. In a study of stomach cancer, high expression of EGF, betacellulin, epiregulin, heparin-binding epidermal growth factor-like growth factor, TGFα, and AREG was detected in 8%, 12%, 24%, 29%, 31%, and 48% of the cases, respectively, and there are considerable positive correlations among the ligands (Byeon et al., 2017). Hobor et al. showed that CRC cells resistant to EGFR mAbs
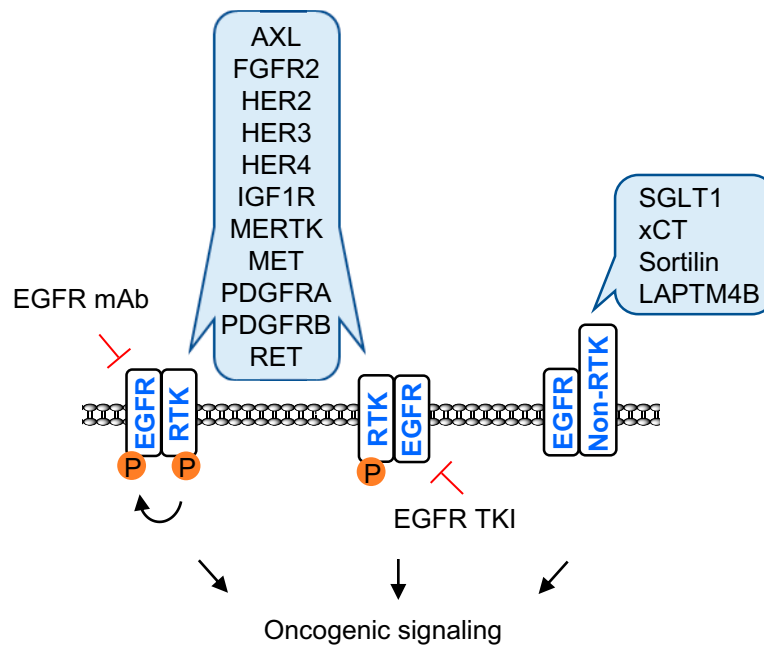
Fig. 2. EGFR heterodimers that may render EGFR inhibitors ineffective. Listed are RTKs and non-RTK membrane proteins that have been shown to heterodimerize with EGFR, most of which have been shown to confer resistance to EGFR inhibitors. The RTKs that bind to EGFR may transphosphorylate EGFR, thereby rendering EGFR mAbs ineffective, and may autophosphorylate themselves, thereby rendering EGFR TKIs ineffective. Notably, unlike other proteins that bind to EGFR to promote oncogenic signaling, sortilin binds to EGFR to inhibit its signaling by inducing its internalization, but sortilin may be downregulated in cancer cells. lysosomal-associated transmembrane protein 4B binds to EGFR in the endosome.

secret TGFα and AREG to protect surrounding cells from EGFR blockade (Hobor et al., 2014). In addition, EGFR kinase may be activated without ligand binding. EGFR becomes tyrosine phosphorylated and initiates signaling when overexpressed, while subsequent ligand binding may induce EGFR conformation change and a shift of downstream signaling (Chakraborty et al., 2014). Palmitoylation of EGFR by intracellular fatty acid synthase also leads to EGFR dimerization and kinase activation in the absence of ligand (Bollu et al., 2015). Persistent signaling by mutated EGFR in TKI-resistant cancer cells was reported to rely on EGFR palmitoylation (Ali et al., 2018). It was also shown that Janus tyrosine kinase 2 (JAK2) and SRC activate EGFR by phosphorylating its tyrosine residues (Yamauchi et al., 1997; Biscardi et al., 1999). Most notably, constitutive activation of EGFR kinase results from activating gene mutation, which is common in NSCLC and GBM as mentioned before. However, while EGFR overexpression and mutation are known to drive oncogenesis and confer therapy resistance, it is not known to what extent EGFR activation by fatty acid synthase, Janus tyrosine kinase 2, or SRC may contribute to these activities.

B. Epidermal Growth Factor Receptor Internalization and Trafficking

Activated EGFR may be internalized, which may be recycled back to cell membrane or delivered to lysosome (Sigismund et al., 2008), mitochondria (Demory et al., 2009), nucleus (Wang et al., 2010), or exosome (Sanderson et al., 2008) (Fig. 1). The mechanisms of EGFR internalization and trafficking are complex and are not fully known. Sortilin, a membrane glycoprotein, was shown to bind to EGFR (Fig. 2) and limit its signaling by promoting its internalization in lung cancer (Al-Akhrass et al., 2017). In NSCLC patients, sortilin expression decreases with increase in pathologic grade and strongly correlates with survival, especially in patients with high EGFR expression (Al-Akhrass et al., 2017). EGFR is internalized by clathrin-mediated endocytosis (CME) or non-clathrin endocytosis as a function of ligand dose and EGFR ubiquitination, with high ligand dose and lack of ubiquitination favoring CME (Sigismund et al., 2005, 2013). p38 kinase-mediated phosphorylation of the C-terminal tail of unliganded EGFR, which may result from minimal EGFR activation by low level of ligand, also induces EGFR internalization via CME (Tanaka et al., 2018). CME-internalized EGFR is predominantly recycled back to cell membrane, whereas non-clathrin endocytosis commits EGFR to degradation in lysosomes (Sigismund et al., 2008). There is evidence that CME contributes to cancer resistance to EGFR TKI (Ménard et al., 2018; Kim et al., 2021). Additional information is available in a comprehensive review (Caldieri et al., 2018), regarding the mechanism of EGFR endocytosis, postendocytic trafficking, importance of endocytosis in controlling EGFR signaling and function, and how cancer cells evade endocytic control of EGFR singling to gain growth advantage. Interestingly, genetic and pharmacologic dynamin-mediated inhibition of EGFR endocytosis was shown to improve natural

killer cell-mediated antibody-dependent cellular cytotoxicity (ADCC) and reverse tumor cell resistance to cetuximab (Chew et al., 2020).

C. Epidermal Growth Factor Receptor Functions in Noncanonical Locations

While recycling EGFR back to cell membrane helps sustain its signaling, EGFR trafficked to other locations may also exert oncogenic activities. Nuclear EGFR has been shown to promote cancer growth, progression, and therapy resistance via multiple mechanisms (Brand et al., 2011; Lee et al., 2015a). Nuclear EGFR functions as a transcriptional coactivator for oncogenes (e.g., cyclin D1 and cyclooxygenase-2), and as a protein kinase that phosphorylates, stabilizes, and activates proliferation cell nuclear antigen and ataxia-telangiectasia mutated protein kinase. It also physically interacts with DNA-dependent protein kinase to promote DNA repair. EGFR may also contribute to cancer growth, progression and therapy resistance by binding to p53 upregulated modulator of apoptosis to prevent its accumulation in mitochondria and by translocating to mitochondria to induce mitochondrial fission and distribution (Zhu et al., 2010; Che et al., 2015). EGFR shed from cancer cells and carried in exosomes was shown to induce angiogenic signaling in endothelial cells (Al-Nedawi et al., 2009). Some of the EGFR functions in the noncanonical locations may be kinase-independent, such as its transcriptional functions, and its regulation of DNA-dependent protein kinase and p53 upregulated modulator of apoptosis. Even if the EGFR functions are kinase-mediated in these locations, it is not known if any clinically used EGFR inhibitor has an effect on such functions.

III. Kinase-Independent Activities of Epidermal Growth Factor Receptor

EGFR knockout either is embryonically lethal or causes the newborn to die within 3 weeks, depending on the genetic background (Sibilia and Wagner, 1995; Threadgill et al., 1995). However, mice carrying a point mutation in the EGFR kinase domain (V743G) which reduces its tyrosine kinase activity by 80% to 95%, are normal except for some abnormalities in the skin and eye (Luetteke et al., 1994). A kinase inactive EGFR mutant (D813A) stimulates MAP kinase activity and DNA synthesis in response to EGF in cultured cells (Coker et al., 1994). Another kinase inactive EGFR mutant (K721R) prevents apoptosis induced by interleukin 3 withdrawal (Ewald et al., 2003). Kinase-inactive EGFR mutants also were detected in human NSCLC tumors (Kancha et al., 2009). There is evidence that EGFR exerts its kinase-independent functions in several cellular locations, including cell membrane, endosome, mitochondria, and nucleus. On cell membrane, loss of EGFR expression, but not inhibition of its kinase activity, results in autophagic cancer cell death (Weihua et al., 2008). EGFR, independent of its kinase activity,

may prevent cell death in part by directly interacting with sodium/glucose cotransporter 1 (Fig. 2), which stabilizes sodium/glucose cotransporter 1 and maintains intracellular glucose (Weihua et al., 2008). Independent of its kinase activity, EGFR also increases cancer cell invasion by directly interacting with and promoting the expression and function of the cysteine-glutamate transporter xCT (Fig. 2) (Tsuchihashi et al., 2016). xCT is a major cell membrane antiporter that mediates cellular uptake of cysteine and has been implicated in tumor growth, progression, and drug resistance (Liu et al., 2020). At the endosome, EGFR interacts with the lysosomal-associated transmembrane protein 4B (Fig. 2), independent of its kinase function, to stimulate autophagy for survival under serum starvation or metabolic stress (Tan et al., 2015). Notably, autophagy may have opposing and context-dependent effects on cancer cell growth and survival (Yun and Lee, 2018). Mitochondrial EGFR was shown to induce mitochondria fission independent of its kinase activity (Che et al., 2015). Nuclear EGFR may also exert its transcriptional activity independent at least partly of its kinase activity. Kinase-deficient EGFR was shown to transcriptionally activate *FOS* gene expression (Eldredge et al., 1994). In addition, as described in Section V, EGFR may function without requiring its kinase activity by forming heterodimeric signaling units with other RTKs. Additional information about the kinase-independent activities of EGFR may be found in previous reviews (Lee et al., 2015a; Tan et al., 2016; Sigismund et al., 2018; Thomas and Weihua, 2019). Collectively, while the literature on the kinase-independent functions of EGFR is much less than that on the kinase-dependent functions of EGFR, there is convincing evidence that EGFR exerts significant kinase-independent functions.

IV. Mechanisms of Actions And Therapeutic Activities of Clinically Used Epidermal Growth Factor Receptor Inhibitors

A. Tyrosine Kinase Inhibitors

Sixteen EGFR TKIs have been approved for clinical use (Table 1). Some of the EGFR TKIs are reversible inhibitors, including brigatinib, elotinib, gefitinib, icotinib, lapatinib, simotinib, and vandetanib. Others are irreversible inhibitors, including afatinib, almonertinib, dacomitinib, lazertinib, mobocertinib, neratinib, olmutinib, osimertinib, and pyrotinib. The reversible inhibitors function by competing with ATP binding to the kinase domain, whereas the irreversible inhibitors act by covalently binding to a cysteine residue (Cys797) in the kinase domain (Hossam et al., 2016). The TKIs differ in target specificity with regard to wild-type (WT) EGFR versus its mutants. For example, gefitinib and erlotinib are as potent against WT EGFR as against sensitive mutants, including exon 19 deletion (Exon19del), L858R, and L861Q (Kitagawa et al., 2013). Osimertinib, however, is 7.5 to 300 times more

potent against EGFR mutants, including L858R/T790M, Exon19del/T790M, Exon19del, L858R, and L861Q, than WT EGFR, with IC_{50} values of 0.1, 0.9, 0.93, 1, 4, and 30 nM, respectively (Han et al., 2021b). The EGFR TKIs have been approved only for treating NSCLC harboring mutant EGFR, except for several TKIs that also target other RTKs and are used to treat HER2-positive breast cancer or medullary thyroid cancer (Table 1). While NSCLC patients whose tumors harbor EGFR mutation frequently derive clinical benefit from EGFR TKI, resistance invariably develops, typically after a median of 9 to 15 months of treatment (Westover et al., 2018; Leonetti et al., 2019). Also, 20% to 30% of the patients are intrinsically resistant to the drugs, i.e., no response or responding for less than 3 months (Wang et al., 2016; Santoni-Rugiu et al., 2019). The drug resistance mechanisms are complex and not fully known. The known resistance mechanisms include EGFR mutation or amplification; activation of other RTKs, such as AXL, HER2, insulin like growth factor 1 receptor (IGF1R), and MET; mutation of KRAS and PIK3CA; epithelial-mesenchymal transition; and transformation to small cell lung cancer (Wang et al., 2016; Westover et al., 2018; Leonetti et al., 2019; Santoni-Rugiu et al., 2019; Shi et al., 2022a). The key vulnerability of drug-resistant NSCLC cells is unknown.

Notably, a new class of EGFR TKIs, known as allosteric inhibitors, have shown promising preclinical activities against therapy-resistant EGFR mutants, including those with T790M and/or C797S mutations (Jia et al., 2016; De Clercq et al., 2019; Gero et al., 2022; Obst-Sander et al., 2022; To et al., 2022). Notably, C797S mutant is resistant to clinically available EGFR TKIs. There is evidence that allosteric EGFR inhibitors and other EGFR inhibitors bind to target cooperatively (Beyett et al., 2022). Studies have also shown that combining an allosteric EGFR inhibitor with osimertinib or cetuximab is more effective than any single agent (Jia et al., 2016; To et al., 2019; Obst-Sander et al., 2022). However, homodimerization or heterodimerization of EGFR mutants with HER family member confers resistance to allosteric EGFR inhibitors (To et al., 2019, 2022).

B. Monoclonal Antibodies

Four EGFR mAbs have been approved for clinical use (Table 1), three of which are approved in the United States, including cetuximab, panitumumab, and necitumumab. Cetuximab is approved for both CRC and HNSCC. Panitumumab is approved for CRC, and necitumumab is approved for squamous NSCLC. More than 80% of primary and metastatic CRCs are EGFR-positive, with overexpression in about 60% of the cases (Shia et al., 2005; Spano et al., 2005). EGFR mutation in CRC is uncommon, although several rare mutations in the ECD may prevent binding of cetuximab and/or panitumumab (Arena et al., 2015; Price et al., 2020). Both cetuximab and panitumumab bind to subdomain 3 of EGFR ECD to block EGFR

activation by ligands (Li et al., 2005; Sickmier et al., 2016). Cetuximab is an IgG1 mAb and therefore can also activate ADCC (Kimura et al., 2007), whereas panitumumab is an IgG2 mAb and is incapable of eliciting ADCC. However, the two mAbs show similar efficacy in CRC patients (Price et al., 2014). Only about 10% of chemotherapy refractory patients respond to mAb monotherapy, and response (median progression-free survival) lasts about 1.5 to 3.5 months, although combination with chemotherapy increases treatment response (Cunningham et al., 2004; Saltz et al., 2004; Hecht et al., 2007; Van Cutsem et al., 2007). Likewise, cetuximab monotherapy produces partial response in only 8% to 11% of HNSCC patients (Fury et al., 2012). Adding cetuximab to chemotherapy in HNSCC increases response rate from 20% to 36%, median progression-free survival from 3.3 to 5.6 months, and median overall survival from 7.4 to 10.1 months (Vermorken et al., 2008). Necitumumab is an IgG1 mAb and also inhibits EGFR by binding to subdomain 3 of its ECD (Li et al., 2008). It is active in squamous carcinoma but not adenocarcinoma in NSCLC, at least partly because the EGFR mutation rate is very low in the former (2.1%–4.5%) (Cheung et al., 2020; Joshi et al., 2017) and EGFR overexpression is more common in squamous NSCLC (82%) than in nonsquamous NSCLC (40%) (Hirsch et al., 2003). However, even in squamous NSCLC, necitumumab efficacy is very limited. Adding necitumumab to chemotherapy increased median overall survival only from 9.9 to 11.5 months (Thatcher et al., 2015). Nimotuzumab is an IgG1 mAb and also binds to subdomain 3 of EGFR ECD (Talavera et al., 2009), which has not been approved in the United States. In a randomized phase 2 trial in NSCLC patients in India, complete response and partial response were 3.6% and 50% in nimotuzumab plus chemotherapy, respectively, and 4% and 30.9% in the chemotherapy control, respectively (Babu et al., 2014). Nimotuzumab did not significantly impact median progression-free survival or overall survival. As with EGFR TKIs, the mechanisms that confer primary and acquired resistance to the EGFR mAbs, which were uncovered mainly from studies in CRC, are complex and not fully known. The known resistance mechanisms include activating mutations of KRAS, NRAS, BRAF, and PIK3CA; loss of PTEN; activation of VEGFR1 and IGF1R; amplification of HER2 and MET; mutation or methylation of EGFR; overexpression of EGFR ligand; and epithelial-mesenchymal transition (Van Emburgh et al., 2014; Bardelli and Siena, 2010; Zhou et al., 2021; Park et al., 2022). The key vulnerability of drug-resistant NSCLC cells is unknown.

C. CIMAvax

CIMAvax is currently undergoing clinical evaluation in the United States in NSCLC patients, and its therapeutic efficacy has not been reported yet. A previous phase 3 trial in NSCLC patients in Cuba

showed that CIMAvax induces anti-EGF antibodies and decreases serum EGF level, as expected but only increases median survival time from 8.86 months in the control arm to 10.83 months in the vaccine arm (Rodriguez et al., 2016). The limited efficacy is not surprising, however, because other EGFR ligands may compensate for EGF loss (Fig. 1), and activating mutation of EGFR may render it independent of EGF or other ligands.

V. Sustained Epidermal Growth Factor Receptor Overexpression and Epidermal Growth Factor Receptor Crosstalk with Other Receptor Tyrosine Kinases to Counter Epidermal Growth Factor Receptor Inhibitors

A. Lack of Downregulation of Epidermal Growth Factor Receptor and Its Mutants Confers Resistance to Epidermal Growth Factor Receptor Inhibitors

In cancer cells that overexpress WT or mutated EGFR, the expression level of the proteins often remains high when treated by EGFR TKIs or after developing resistance to these agents, while its autophosphorylation may be inhibited (Wood et al., 2004; Tabara et al., 2012; Jacobsen et al., 2017; Liu et al., 2018; Thomas et al., 2019; Shaurova et al., 2020). Shtiegman et al. reported that EGFR mutants that are associated with NSCLC, such as L858R/T790M, may dimerize with HER2 to evade ubiquitination and subsequent degradation (Shtiegman et al., 2007). Ménard et al. showed that reactivation of lysosomal degradation of mutant EGFR in NSCLC cells, including L858R/T790M and other mutants, by inhibiting clathrin overcomes resistance to EGFR TKIs (Ménard et al., 2018). An EGFR degrader known as DPBA (a 23-hydroxybetulinic acid derivative) is more effective than EGFR TKIs, including gefitinib, afatinib, and osimertinib, in inhibiting the growth of NSCLC cells expressing WT or mutated EGFR (Yao et al., 2020). Targeted degradation of EGFR and HER2 by proteolysis targeting chimera (PROTAC) is also more effective in inhibiting cancer cell growth than inhibiting the kinase activity of the RTKs (Burslem et al., 2018). Failure to downregulate EGFR by mAbs may also be a critical cause for resistance to these agents. Pre-treatment tumor EGFR level does not correlate with clinical response to cetuximab and panitumumab in CRC (Cunningham et al., 2004; Hecht et al., 2010). However, EGFR downregulation after treatment with each mAb predicts the antitumor effect (Okada et al., 2017). In the majority of cultured cell lines and mouse tumor models that are either sensitive or resistant to cetuximab, panitumumab, or necitumumab, the mAbs are incapable of downregulating EGFR or even increasing its expression as well as increasing the expression of its family members, while its tyrosine phosphorylation may be inhibited (Wheeler et al., 2008; Ashraf et al., 2012; Misale et al., 2012; Iida et al., 2013; Iida et al., 2014;

Troiani et al., 2014; Ohnishi et al., 2015; Bagchi et al., 2018; Yang et al., 2022). However, in cell lines and tumors where cetuximab downregulates EGFR or its mutants, it invariably inhibits the growth of these cells and tumors, whether they express WT or mutated EGFR (Perez-Torres et al., 2006; Yang et al., 2022). Also, downregulation of EGFR by siRNA or targeted degradation of EGFR by PEPD^{G278D}, a recombinant human protein that is discussed later in detail, inhibits CRC cells that are resistant to cetuximab and panitumumab (Yang et al., 2022). It is poorly understood as to why EGFR mAbs downregulate EGFR in some cancer cells but not in most other cancer cells. Wheeler et al. showed that EGFR insensitivity to cetuximab may result from dysregulation of EGFR internalization and degradation involving CBL, an E3 ligase (Wheeler et al., 2008). Liao et al. showed that EGFR methylation in its ECD renders it less sensitive to cetuximab (Liao et al., 2015). Several rare acquired mutations in the ECD of EGFR were reported in CRC patients following cetuximab treatment, including R451C, K467T, and S492R, each of which prevents cetuximab binding and confers resistance to cetuximab (Arena et al., 2015; Price et al., 2020). The R451C and K467T mutants also bind poorly to panitumumab (Arena et al., 2015).

B. Heterodimerization of Epidermal Growth Factor Receptor and Its Mutants with Other Receptor Tyrosine Kinases Confers Resistance to Epidermal Growth Factor Receptor Inhibitors

EGFR is well known to heterodimerize with all its family members, including HER2, HER3, and HER4 (Okines et al., 2011). It also heterodimerizes with other RTKs, including AXL, IGF1R, MET, fibroblast growth factor receptor 2, MER receptor tyrosine kinase, platelet derived growth factor receptor α, platelet derived growth factor receptor β, and RET (Fig. 2) (Morgillo et al., 2006; Tanizaki et al., 2011; Chang et al., 2015; Wang et al., 2015; Chakravarty et al., 2017; Ortiz-Zapater et al., 2017; Taniguchi et al., 2019; Yan et al., 2022). AXL, HER2, IGF1R, MER receptor tyrosine kinase, MET, and RET fusion have been shown to confer resistance to EGFR TKIs in NSCLC (Yonesaka et al., 2011; Yeo et al., 2015; Piotrowska et al., 2018; Taniguchi et al., 2019; Marrocco et al., 2021; Zhu et al., 2021a; Yan et al., 2022). More information about EGFR heterodimerization may be found in a previous review (Kennedy et al., 2016). HER2 has also been shown to heterodimerize with a variety of RTKs (Kennedy et al., 2019). The RTKs mentioned above likely render cancer cells resistant to EGFR inhibitors at least in part by forming heterodimeric signaling units with EGFR, to allow EGFR to continue to exert oncogenic activities despite suppression of its tyrosine kinase. This property is not unique to EGFR, as HER3 is kinase-defective but exerts strong oncogenic activity by heterodimerizing with other RTKs as described before. Moreover, HER2, HER3, and platelet derived growth factor receptor β have been shown to inhibit EGFR

endocytosis by heterodimerizing with it (Wang et al., 1999; Wang et al., 2015). Thomas et al. showed that EGFR TKIs, including gefitinib, erlotinib, and AEE728, stimulate EGFR dimerization, that EGFR TKI-inhibited EGFR is still required for the survival of EGFR-expressing cancer cells, and that downregulation of EGFR by siRNA or herdegradin (a peptide) kills TKI-resistant cells (Thomas et al., 2019). In NSCLC cells that harbor EGFR Exon19del (E746-A750del), EGFR TKI osimertinib stimulates AXL and increases AXL association with the EGFR mutant (Taniguchi et al., 2019). EGFR E746-A750del also heterodimerizes with HER2 and HER3 in NSCLC cells (Sakai et al., 2007). Using computation methods, Zhu et al. found that there is a looser EGFR crosstalk with MET, HER2, and IGF1R for the drug-sensitive EGFR mutant (L858R) than for the drug-resistant mutant (L858R/T790M) (Zhu et al., 2021b). Indeed, more MET binds to EGFR L858R/T790M than to EGFRL858R and WT EGFR (Ortiz-Zapater et al., 2017). In cancer cells that develop acquired resistance to cetuximab, there is also increased EGFR dimerization with HER2 and HER3 and activation of these RTKs (Wheeler et al., 2008). These findings strongly suggest that disrupting EGFR heterodimerization may be key to improving the efficacy of EGFR targeted therapies and also suggest that it may not be sufficient to disrupt just one type of EGFR heterodimer.

VI. Emerging Epidermal Growth Factor Receptor Degraders and Their Therapeutic Activities

EGFR exerts both kinase-dependent and -independent oncogenic activities, and various EGFR heterodimers may contribute to the kinase-independent activities of EGFR and confer drug resistance, as described before. Therefore, eliminating the physical presence of EGFR may be a much more effective therapeutic strategy than inhibiting its kinase activity. Many agents have been shown to induce EGFR degradation, including PROTACs, non-PROTAC small molecules, antibody combinations, and non-antibody proteins (Table 2). These agents have shown promising preclinical therapeutic activities. Some of the degraders may primarily target cell surface EGFR, but they may also abolish EGFR functions in other locations (exosome, endosome, mitochondria, and nucleus), as EGFR is transferred to these locations from cell surface membrane.

A. Proteolysis Targeting Chimeras

PROTAC works by linking a small molecule that binds to a target protein with a ligand for an E3 ligase and achieving target degradation via intracellular proteolysis by the ubiquitin-proteasome system. It has emerged as a promising new platform for cancer drug development. Many EGFR-directed PROTACs have been synthesized (Table 2) and evaluated in cultured cells, one of which was also evaluated in a mouse tumor model in vivo. Burslem et al. synthesized several PROTACs by conjugating an EGFR TKI to a ligand that binds to E3 ligase von Hippel-Lindau (VHL) (Burslem et al., 2018). Using lapatinib as the EGFR TKI which binds to both EGFR and HER2, they showed that the PROTAC induces the degradation of both RTKs and is more effective in inhibiting cell growth than the equivalent kinase inhibitor. Using gefitinib, which binds to EGFR mutants, they showed that the PROTAC induces the degradation of EGFR mutants including Exon19del and L858R. Exon19del and L858R mutants as well as other EGFR kinase domain mutants described later are activating EGFR mutants that occur in NSCLC. Using afatinib, they showed that the PROTAC induces the degradation of gefitinib-resistant $EGFR^{L858R+T790M}$. Zhang et al. synthesized a PROTAC that specifically targets

TABLE 2
Emerging degraders of EGFR and its family members

Agent class	Agent	Targets	References
PROTACs	Lapatinib-based	EGFR, HER2	Burslem et al., 2018
	Dacomitinib-based	$EGFR^{Exon19del}$	Zhang et al., 2020a; Shi et al., 2022b
	Novel TKI-based		
	Gefitinib-based	$EGFR^{Exon19del}$, $EGFR^{L858R}$	Burslem et al., 2018; Cheng et al., 2020; Yu et al., 2022
	Gefitinib derivative-based		
	Afatinib-based	$EGFR^{L858R+T790M}$	Burslem et al., 2018; Zhang et al., 2020b
	Novel TKI-based		
	Osimertinib-based	$EGFR^{Exon19del}$, $EGFR^{L858R+T790M}$	He et al., 2020; Zhao et al., 2020; Qu et al., 2021
	Novel TKI-based		
	Novel TKI-based	$EGFR^{L858R+T790M}$, $EGFR^{L858R+T790M+C797S}$, $EGFR^{L858R+T790M+C718Q}$	Jang et al., 2020
	Novel TKI-based	$EGFR^{Exon19del+T790M+C797S}$	Zhang et al., 2022
Non-PROTAC small molecules	DPBA	EGFR, $EGFR^{E736-A750del}$, $EGFR^{L858R+T790M}$	Yao et al., 2020
	Hydroxytyrosol	EGFR	Terzuoli et al., 2016
	VM26 and analogs	EGFR	Iradyan et al., 2019
	Tephrosin	EGFR, HER2, HER3	Choi et al., 2010
Antibody combinations	Sym004	EGFR	Pedersen et al., 2010
	Sym013	EGFR, HER2, HER3	Jacobsen et al., 2015
Non-antibody proteins	PEPD, $PEPD^{G278D}$	EGFR, HER2	Yang et al., 2014, 2015, 2016

The EGFR mutants listed in this table occur in NSCLC.

$EGFR^{L858R/T790M}$ by conjugating an experimental $EGFR^{L858R/T790M}$-specific inhibitor to a ligand that binds to VHL and showed that it induces the degradation of $EGFR^{L858R/T790M}$ but not WT EGFR (Zhang et al., 2020b). Zhao et al. showed that a PROTAC that links an experimental EGFR TKI, 9-cyclopentyl-8-phenylamino-2-(4-(piperazin-1-yl)phenylamino)-9H-purine, to a ligand for VHL is highly effective against $EGFR^{Exon19del}$ and $EGFR^{L858R+T790M}$ but not WT EGFR (Zhao et al., 2020). Several other PROTACs, linking a novel EGFR TKI to a ligand for either VHL or cereblon, another E3 ligase, degrade $EGFR^{Exon19del}$ but have weak activity against $EGFR^{L858R+T790M}$ and WT EGFR (Zhang et al., 2020a). He et al. generated a PROTAC by conjugating osimertinib to a ligand for cereblon, which significantly decreases the expression of $EGFR^{Exon19del}$ and $EGFR^{L858R+T790M}$ (He et al., 2020). Cheng et al. generated PROTACs by conjugating a gefitinib derivative to a ligand for either VHL or cereblon and showed that the PROTACs potently induce the degradation of $EGFR^{Exon19del}$ and $EGFR^{L858R}$ but not WT EGFR (Cheng et al., 2020; Yu et al., 2022). Qu et al. synthesized PROTACs by conjugating canertinib, an experimental EGFR TKI, or a derivative of it to a ligand for cereblon and showed that these agents degrade $EGFR^{L858R+T790M}$ and $EGFR^{Exon19del}$ but is inactive against $EGFR^{Exon19del+T790M}$ and WT EGFR (Qu et al., 2021). Shi et al. synthesized PROTACs by conjugating dacomitinib to a ligand for cereblon or VHL and showed that these agents are highly effective against $EGFR^{Exon19del}$ but not WT EGFR, one of which strongly inhibits the growth of NSCLC cell xenograft harboring $EGFR^{Exon19del}$ in mice (Shi et al., 2022b). Jang et al. generated a PROTAC by conjugating an allosteric EGFR inhibitor to a ligand for cereblon and showed that it is effective against multiple EGFR mutants, including $EGFR^{L858R+T790M}$, $EGFR^{L858R+T790M+C797S}$, and $EGFR^{L858R+T790M+C718Q}$, but not WT EGFR (Jang et al., 2020). Zhang et al. showed that an allosteric EGFR inhibitor-derived VHL-recruiting degrader is effective against $EGFR^{Exon19del+T790M+C797S}$ but is only weakly effective or not effective at all against WT EGFR, $EGFR^{Exon19del}$, $EGFR^{L858R+T790M}$, and $EGFR^{L858R}$ (Zhang et al., 2022). These studies show the widespread interest in developing PROTACs targeting EGFR, its mutants, and HER2 in cancer and the promise of this approach. However, there is significant redundancy in generating PROTACs targeting EGFR mutants by different research groups, and it is unclear whether any agent will be effective against a broad spectrum of EGFR mutants occurring in NSCLC.

B. Non-Proteolysis Targeting Chimera Small Molecules

Yao et al. found that a 23-hydroxybetulinic acid derivative, termed DPBA, is an EGFR degrader by screening more than 700 natural compounds and their derivatives (Yao et al., 2020). DPBA induces the degradation of EGFR but not its family members, including HER2, HER3, and HER4. It induces lysosomal degradation of both WT EGFR and mutants including E736-A750del and L858+T790M by binding to their ECD and inducing clathrin-independent endocytosis, without inducing their dimerization. It inhibits the growth of cancer cells and tumors harboring WT EGFR, $EGFR^{E736-A750del}$, or $EGFR^{L858R+T790M}$ in vitro and in vivo. Terzuoli et al. reported that hydroxytyrosol, from olive oil, induces EGFR degradation by stimulating its ubiquitination and inhibiting the growth of EGFR-expressing cancer cells in vitro and in vivo (Terzuoli et al., 2016). Hydroxytyrosol does not appear to downregulate EGFR in normal cells and has not been evaluated against EGFR mutants. Iradyan et al. reported that several furfuryl derivatives of 4-allyl-5-[2-(4-alkoxyphenyl)-quinolin-4-yl]-4H-1,2,4-triazole-3-thiol (VM26 and analogs) inhibit EGFR phosphorylation and induce its internalization and degradation by binding to an allosteric site located in the vicinity of the catalytic pocket in the kinase domain of the receptor (Iradyan et al., 2019). Choi et al. reported that tephrosin, a natural rotenoid, inhibits the phosphorylation of EGFR, HER2, and HER3; induces the internalization and lysosomal degradation of the RTKs; and causes cell death (Choi et al., 2010).

C. Antibody Combinations

Sym004, a 1:1 mixture of two IgG1 antibodies binding to two nonoverlapping epitopes in the ECD subdomain 3 of EGFR, induces rapid internalization and lysosomal degradation of the receptor and is more effective than cetuximab and panitumumab in inhibiting tumor growth in vivo (Pedersen et al., 2010; Jones et al., 2020). Sym004 is also effective against cancer cells and tumors that are resistant to cetuximab and show increased EGFR expression (Iida et al., 2013). Sym004 is also more effective than cetuximab in CRC PDX models but unexpectedly did not improve survival in a phase 2 randomized clinical trial in metastatic CRC with acquired resistance to anti-EGFR mAb (Montagut et al., 2018). It is not known if Sym004 downregulates tumor EGFR in patients. Jacobsen et al. generated a mixture of six antibodies, termed Pan-HER (Sym013), which simultaneously targets EGFR, HER2, and HER3, and showed that Sym013 induces the degradation of all three RTKs and inhibits the growth of cancer cells and tumors in mice, including those resistant to cetuximab and trastuzumab (an anti-HER2 mAb) (Jacobsen et al., 2015; Iida et al., 2016). Notably, HER2 amplification may occur in nearly 4% of patients with metastatic CRC (Dumbrava et al., 2019), and HER2 confers resistance to EGFR inhibition as mentioned before. However, a first-in-human trial showed significant toxicity of Sym013 and potential difficulty in achieving a tolerated regimen with adequate target saturation (Berlin et al., 2022).

D. Non-Antibody Proteins

Peptidase D (PEPD), also known as prolidase, is a widely distributed dipeptidase important for collagen metabolism (Myara et al., 1984). Surprisingly, we found that, while the endogenous intracellular PEPD has no effect on EGFR and its family members, exogenous recombinant human PEPD induces the internalization and lysosomal degradation of both EGFR and HER2 by binding to their ECD (Yang et al., 2013, 2014, 2016, 2019). PEPD binds to ECD subdomain 2 in EGFR but ECD subdomain 3 in HER2 (Yang et al., 2014, 2016). PEPD does not bind to HER3 and HER4 (Yang et al., 2014). The finding that PEPD binds to HER2 at high affinity (Kd = 7 nM) (Yang et al., 2014) was unexpected, because it had been widely believed that HER2 exists in a closed state and cannot be liganded, and no ligand had been previously identified. Crystallography studies showed that the structure of HER2 ECD resembles a ligand-activated conformation that is ready for dimerization (Cho et al., 2003; Garrett et al., 2003). PEPD also represents a novel class of EGFR ligands, as all other EGFR ligands are first synthesized as membrane proteins and harbor an EGF motif, but PEPD neither is a membrane protein nor carries an EGF motif. The binding affinity of PEPD is lower toward EGFR (Kd = 17 nM) than HER2 (Yang et al., 2016). The enzymatic activity of PEPD is not required for targeting EGFR and HER2 (Yang et al., 2013, 2014, 2015, 2016). Recombinant PEPD[G278D], which is enzymatically inactive, shows no difference from PEPD in targeting EGFR and HER2, but it is a more attractive antitumor agent than PEPD. PEPD[G278D] may not interfere with the enzymatic function of endogenous PEPD, and PEPD, but not PEPD[G278D], increases HIF1α growth signaling due to inhibition of its degradation by the products of PEPD enzymatic reaction (Surazynski et al., 2008; Yang et al., 2015). PEPD[G278D] and PEPD have shown strong antitumor activities in preclinical models of EGFR- and/or HER2-overexpressing cancers (Yang et al., 2014, 2015, 2016). In models of HER2-positve breast cancer and EGFR-positive CRC, PEPD[G278D] strongly inhibits the growth of cancer cells and tumors that are resistant to clinically used EGFR and HER2 inhibitors (Yang et al., 2019, 2022). PEPD[G278D] and PEPD target EGFR and HER2 overexpressed in cancer cells but not the RTKs expressed low in normal cells (Yang et al., 2015; 2019). This apparently is due to their unique binding mode. PEPD[G278D] as well as PEPD are homodimers, and each subunit binds to a monomer of EGFR or HER2 to form a tetra-complex (Yang et al., 2014, 2015, 2019), which requires the RTKs to be overexpressed on cell membrane. Furthermore, while PEPD[G278D] binds to both EGFR and HER2, it does not bind to both RTKs simultaneously and disrupts EGFR-HER2 heterodimers by forming a tetra-complex with each RTK (Yang et al., 2015). This may be due to PEPD[G278D] binding to different locations in the ECD of the RTKs. Besides WT EGFR and HER2, PEPD[G278D] also targets EGFR mutants that do not bind to cetuximab and panitumumab (Yang et al., 2022). It is possible that PEPD[G278D] is also effective against a wide spectrum of EGFR and HER2 mutants that occur in NSCLC, since PEPD[G278D] binds to the ECD of the RTKs but the mutations occur in the intracellular kinase domain.

VII. Concluding Remarks

EGFR drives cancer development and progression through both kinase-dependent and kinase-independent functions. EGFR inhibitors that are currently available in the clinic, including mAbs and TKIs, inhibit EGFR kinase without inhibiting or even promoting its kinase-independent functions. These inhibitors have shown clinical efficacy in only a few types of cancers, and even in these cancers, drug resistance is common and treatment efficacy is not durable. CIMAvax is also limited in its mechanism of action for inhibiting EGFR, as it indirectly inhibits EGFR tyrosine kinase by inducing EGF-neutralizing antibody, but other EGFR ligands may compensate for EGF loss. CIMAvax is not known to modulate the kinase-independent activity of EGFR, and available data show very limited clinical efficacy of this vaccine. There is accumulating evidence that the kinase-independent activities of EGFR must also be targeted in addition to inhibiting its tyrosine kinase activity to achieve better therapeutic outcomes and minimize drug resistance. Targeting the degradation of EGFR is a promising strategy for simultaneously abolishing both kinase-dependent and -independent functions of the RTK. A number of new agents have shown promising preclinical activity in inducing EGFR degradation and inhibiting cancer cell growth. Some of the agents also induce the degradation of other EGFR family members, which likely enhances the therapeutic efficacy. It will be important to confirm that the target is overexpressed in cancer cells before use of such agent. Tumor heterogeneity undoubtedly presents challenge to any targeted therapy, and further research is needed to determine the therapeutic efficacy of targeted degradation of EGFR and its family members in tumors that carry other drug resistance drivers. Notably, we showed that KRAS mutation (G12D or G13D), BRAF mutation (V600E), and/or PIK3CA mutation (P449T or H1047R) do not confer resistance of CRC cells and tumors to PEPD[G278D], which induces the degradation of both EGFR and HER2 (Yang et al., 2022). It is also important to evaluate whether combination of an EGFR degrader with other antitumor agents enhances treatment efficacy.

Data Availability

The author declares that all the data supporting the findings are contained within the paper.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Zhang.

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