Oncogene (2014) **33,** 1073–1081

www.nature.com/onc

REVIEW

Signaling cross-talk in the resistance to HER family receptor targeted therapy

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Epidermal growth factor receptor (EGFR) and human EGFR 2 (HER2) have an important role in the initiation and progression of various types of cancer. Inhibitors targeting these receptor tyrosine kinases are some of the most successful targeted anticancer drugs widely used for cancer treatment; however, cancer cells have mechanisms of intrinsic and acquired drug resistance that pose as major obstacles in drug efficacy. Extensive studies from both clinical and laboratory research have identified several molecular mechanisms underlying resistance. Among them is the role of signaling cross-talk between the EGFR/HER2 and other signaling pathways. In this review, we focus particularly on this signaling cross-talk at the receptor, mediator and effector levels, and further discuss alternative approaches to overcome resistance. In addition to well-recognized signaling cross-talk involved in the resistance, we also introduce the cross-talk between EGFR/HER2-mediated pathways and pathways triggered by other types of receptors, including those of the Notch, Wnt and TNFR/IKK/NF-κB pathways, and discuss the potential role of targeting this cross-talk to sensitize cells to EGFR/HER2 inhibitors.

Oncogene (2014) **33,** 1073–1081; doi:10.1038/onc.2013.74; published online 1 April 2013

Keywords: signaling cross-talk; EGFR; HER2; drug resistance

INTRODUCTION

Receptor tyrosine kinases (RTKs) are cell-surface transmembrane proteins that consist of an extracellular ligand-binding domain and an intracellular tyrosine kinase domain. The functions of RTKs are essential to normal cell physiology, such as cell proliferation, metabolism and differentiation. Among various RTKs, the epidermal growth factor receptor (EGFR) and the human EGFR 2 (HER2, also known as ErbB2) are frequently upregulated or mutated in a broad spectrum of cancer types and possess aberrant activities that lead to cancer cell proliferation, survival, migration and differentiation.[1,2] For example, *EGFR* is frequently mutated in lung cancer and brain tumors or overexpressed in lung, colon, head and neck, brain, pancreas and breast cancers,[3–6] and HER2 is often overexpressed in breast, gastric, esophageal, pancreatic and ovarian cancers.[7,8]

Regardless of the diverse array of mutations, cancer cells frequently exhibit dependency on a particular signaling pathway that is driven by mutation or overexpression of a single protein. This phenomenon is referred as 'oncogene addiction',[9] a concept that is essential to cancer targeted therapy in the clinical and laboratory research. For instance, lung cancer cells with activated mutation in *EGFR* are dependent on EGFR for their survival, and inhibition of EGFR activity induces drastic cell death and growth arrest in cultured cells and tumor regression in lung cancer patients harboring mutated *EGFR*.[10–12] In contrast, lung cancer patients with wild-type *EGFR* exhibit little or no response to the same treatment, limiting EGFR-targeted therapy only to lung cancer patients with *EGFR* mutation. In general, effective molecularly targeted therapy requires identifying the appropriate predictive biomarkers and selection of patients based on these identified biomarkers, which can substantially increase drug efficacy and improve patient survival.

EGFR tyrosine kinase inhibitors (TKIs, for example, gefitinib and erlotinib) and EGFR monoclonal antibodies (for example, cetuximab and panitumumab) have been approved for clinical usage.[13] Erlotinib is currently used to treat patients with *EGFR*-mutant non-small-cell lung cancer (NSCLC) and pancreatic cancer, and cetuximab is used to treat colon and head and neck cancers with wild-type *K-RAS*. Lapatinib (TKI) and trastuzumab (monoclonal antibodies) are approved for treating HER2-overexpressing breast cancer.[14] While these treatment strategies have significantly improved patient survival, a majority of patients eventually experience disease recurrence when drug resistance develops. To combat resistance, other alternative and more effective combinational treatment strategies based on known molecular mechanisms underlying drug resistance have been investigated in clinical trials.[15] However, the innate and acquired resistance to targeted agents remain the biggest challenges in cancer therapy.

In this review, we provide a brief description of various EGFR and HER2 signaling pathways that are either current or potential drug targets. Furthermore, we highlight the significance of signal cross-talk in resistance to targeted therapy. In addition, we describe some rationally designed therapies that target multiple signaling pathways and have the potential to provide more effective personalized cancer treatment to overcome drug resistance.

KEY SIGNAL TRANSDUCTION FROM RTK TO MAINTAIN CANCER CELL SURVIVAL AND GROWTH

Signal transduction is a series of biochemical modifications of biomolecules, such as proteins, lipids and nucleic acid, through which cells alter intracellular physiology in response to various external stimuli including a number of growth factors and

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Received 25 January 2013; revised 12 February 2013; accepted 12 February 2013; published online 1 April 2013

cytokines. The signals from the extracellular stimuli induce overall alterations in gene expression profiles, resulting in outcomes such as cell proliferation, growth and migration. In the last few decades, the molecular components of the key downstream signaling pathways from the HER family of receptors have been extensively studied, and several key signaling pathways regulating the functions of these receptors have been identified.

Activation of EGFR family proteins is regulated by ligand binding with the exception of HER2, which dimerizes independently of ligands.[16,17] Once dimerization occurs, intracellular tyrosine kinases are fully activated and induce autophosphorylation in their tyrosine residues. These phosphorylated tyrosines function as docking sites for several adapter proteins, such as GRB2 and SHC, which further transduce the signaling pathways through protein–protein interaction and post-translational modifications.[18] Ultimately, EGFR and HER2 activate multiple signaling pathways, including RAS, phosphatidylinositol 3-kinase (PI3K), PKC, Src and signal transducer and activator of transcription 3 (STAT3). Among them, the most investigated downstream pathways in cancer are RAS/RAF/MEK and PI3K/AKT. The key molecules in these signaling pathways, RAS, RAF and PI3K, function as oncogenic proteins and are frequently mutated or activated, even in cancers in which RTKs are not activated.[19–21] Furthermore, cancer cells with *K-RAS* or *PIK3CA* mutation exhibit resistance to RTK inhibitors, but simultaneous inhibition of both PI3K/mammalian target of rapamycin (mTOR) and MEK has been shown to sufficiently induce apoptotic cell death in lung cancers harboring *EGFR* mutant.[22]

The signals from a single RTK activation are amplified at multiple downstream points rather than in a single linear manner. At the receptor level, a phosphorylated RTK recruits and phosphorylates multiple proteins and augment the signaling by each protein, which results in different signal transduction. Major mediator kinases downstream of RTKs also phosphorylate multiple targets to activate or inactivate them, leading to further amplification of the signaling pathways. Downstream effectors including transcription factors and other enzymes then induce multiple target gene expression. Thus, the cross-talk of the signaling from RTKs with many other signaling pathways can occur at various points. To simplify the signaling cross-talk in RTK signaling that may affect drug resistance, we categorized three types of cross-talk at different levels: receptor, mediator and effector (Figure 1). Cross-talk at the receptor level occurs when other types of activated or amplified RTKs, which have the same downstream targets, compensate for the inhibition of targeted RTK. When resistance occurs at the mediator level, constitutive activation or inactivation of mediators due to various mutations or deletions can transduce active signaling independently of RTK. Resistance at effectors level occurs when other signaling pathways alter the activity of critical effectors involved in the survival or cell growth controlled by RTK signaling. In the next sections, we will further introduce the several mechanisms of the resistance to EGFR/HER2 inhibitors that are found in clinical samples and/or experimental systems, and discuss the possible roles of signaling cross-talk.

IDENTIFIED MOLECULAR MECHANISM OF THE RESISTANCE TO EGFR/HER2 INHIBITORS

Resistance through cross-talk at the receptor level

One common mechanism of resistance to EGFR/HER2 inhibitors is the upregulation or activation of alternative RTKs. In *EGFR*-mutant NSCLC patients, these types of acquired TKI resistance arise from the amplification of *MET* and *HER2*, and upregulation of AXL.[23–27] FGFR and IGF-1R activation are also implicated in EGFR TKI resistance in NSCLC.[28,29] In colon cancer, *MET*, *IGF-1R* and *HER2* amplification have been shown to be associated with cetuximab

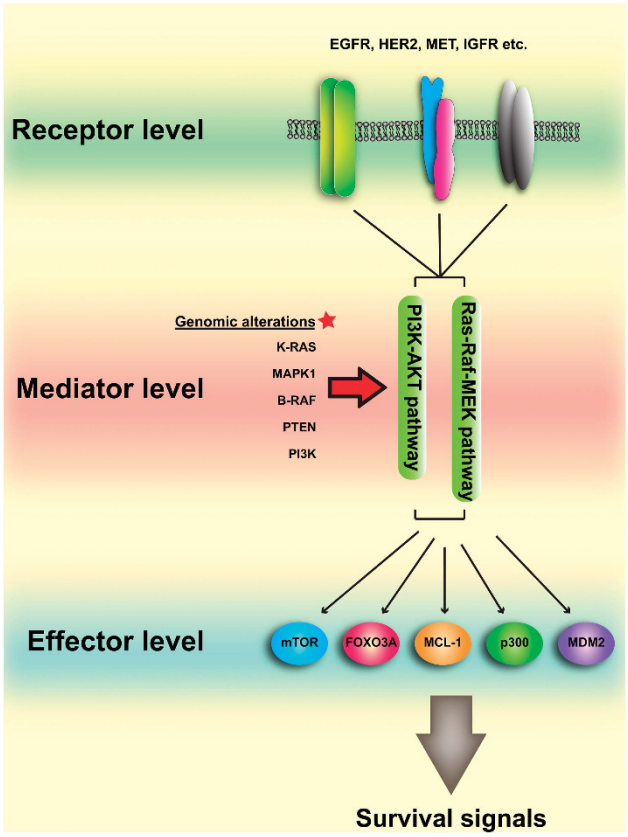

Figure 1. Signaling cross-talk at the various levels of EGFR/HER2 signaling pathways. EGFR/HER2 signaling pathways cross-talk with other signaling pathways mainly at receptor, mediator and effector levels. The cross-talk at the receptor level includes other receptor tyrosine kinases, which have common downstream targets of EGFR/HER2, and affects their signaling pathway. The cross-talk at mediator level includes the activators of key downstream signaling, such as RAS/RAF/MEK/ERK and PI3K/AKT pathways. Multiple genetic alterations of these pathways affect the downstream effectors of EGFR/HER2 in a receptor-independent manner. The cross-talk at the effector level includes a variety of key molecules regulated by EGFR/HER2 signaling. These molecules directly regulate cell survival and growth, and their post-translational modifications are critical for cancer initiation, progression and drug sensitivity.

resistance.[30–32] Trastuzumab resistance in HER2-overexpressing breast cancer cells has been attributed to the activation of EGFR, HER3, EphA2 and IGF-1R.[33–35] In addition to the RTKs overexpression, RTK ligands, such as NRG1, HGF and, FGF, whose expressions are increased through autocrine signals from tumor cells or paracrine signals from stroma, can reverse the sensitivity to EGFR/HER2 TKIs in these receptor-addicted cells.[36] Interestingly, treatment of cancer-related and chemotherapy-induced anemia and fatigue by using human recombinant erythropoietin was shown to confer resistance to trastuzumab in breast cancer cells via Jak2-mediated activation of Src and inactivation of PTEN.[37]

The above-mentioned examples of resistance all belong to cross-talk at the receptor levels (Figure 2). The signaling from alternative RTKs such as MET, IGF-1R, FGFR and EphA2 can maintain the key cell survival and/or proliferation signals in the RAS/RAF/MEK and PI3K/AKT pathways.[23–26,33] Both the pathways can be inhibited by simultaneously blocking EGFR/HER2 and the alternative RTKs in the resistant cells, which results in cancer cell death or growth arrest.[23–26,33] Therefore, simultaneous inhibition of two or more RTKs by combination therapy may be necessary to provide a more effective strategy to overcome this type of drug resistance.

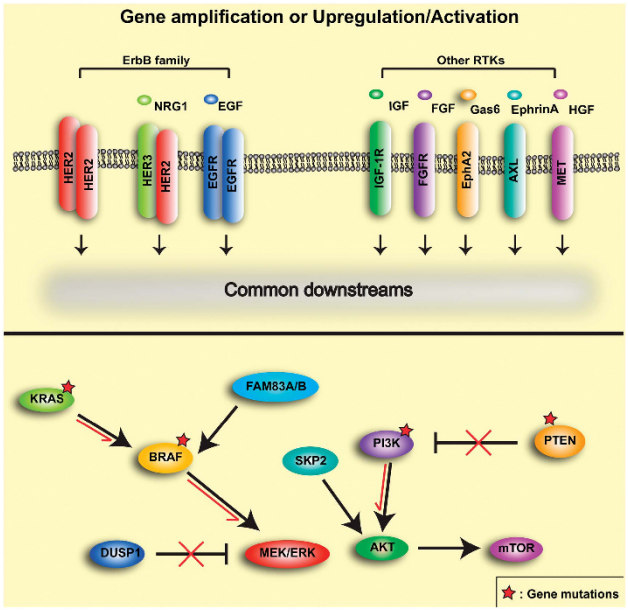

Figure 2. Currently known resistant mechanisms to EGFR/HER2 inhibitors at receptor and mediator levels. The signal cross-talk at the receptor level (top) induces the activation of common downstream pathways of EGFR/HER2, resulting in the drug resistance. This includes activation of MET, AXL and IGF-1R by gene amplification, overexpression or ligands. HER2 and EGFR activation also cause resistance to EGFR and HER2 inhibitors, respectively. HER3 also has a critical role in EGFR/HER2 inhibitors resistance. At the mediator level (bottom), the components of two major pathways (RAS/RAF/MEK/ERK and PI3K/AKT/mTOR) are activated independently of receptors, resulting in the activation of downstream effectors. K-RAS, B-RAF and PI3K are frequently activated by mutation whereas PTEN is inactivated by mutation or deletion in multiple cancer types. Skp2 activates AKT, which is involved in HER2 inhibitor resistance. FAM83A/B activates MEK/ERK and is involved in EGFR TKI resistance.

Resistance through cross-talk at the mediator level

Another common mechanism of resistance involves the direct activation of mediators in the RAS/RAF/MEK or PI3K/AKT pathway (Figure 2). *K-RAS* mutation is most frequently observed mutation in oncogenic drivers. Lung and colon cancer cells harboring mutant *K-RAS* exhibit intrinsic resistance to EGFR inhibitors.[38–40] Interestingly, *K-RAS* mutation is also associated with acquired resistance to cetuximab in colon cancer patients.[41,42] Extracellular signal-regulated kinase (ERK) signaling reactivation by either amplification of *MAPK1* or downregulation of negative regulators of ERK is related to TKI resistance in lung cancer cells.[43] Mutation in *B-RAF* is found in acquired resistance to TKI in lung cancer patients with mutated *EGFR*.[44] In many types of cancer, oncogenic PI3K is frequently activated,[45] and PTEN, a tumor suppressor and negative regulator of PI3K, is often deleted or inactivated.[46] In breast cancer, deletion or mutation of *PTEN* or oncogenic mutation of *PIK3CA* has been shown to associate with resistance to HER2 inhibitors.[47,48] Clinical trial data have demonstrated that PTEN loss is significantly associated with poor trastuzumab response and survival in patients with HER2-positive breast cancer.[49] In *EGFR*-mutated lung cancers, *PIK3CA* mutation is also linked to acquired resistance to EGFR TKI.[50] Interestingly, family with sequence similarity 83 members A and B (FAM83A and FAM83B) have recently been shown to contribute to TKI resistance in breast cancer cells through the upregulation of RAF/MEK/ERK signaling.[51,52] Moreover, the E3 ligase Skp2 SCF complex has also been demonstrated to regulate AKT ubiquitination and activation, and the inhibition of Skp2 can sensitize HER2-overexpressing tumors to trastuzumab *in vivo*.[53]

As mentioned in the previous section, inhibition of oncogenic signaling involved in oncogene addiction is a required and sufficient step to induce apoptosis or cell growth arrest in many types of cancer cells. B-RAF inhibitors are currently used for the treatment of *B-RAF*-mutated melanoma.[54] MEK, PI3K and AKT inhibitors are also being tested in clinical trials to treat multiple cancer types and have the potential to effectively overcome resistance induced by the activation of RAS/RAF/MEK or PI3K/AKT pathway. Indeed, some preclinical studies have shown that the combination of EGFR and MEK inhibitors reduces the occurrence of EGFR TKI resistance,[43] and that inhibition of the PI3K pathway restores sensitivity to HER2 inhibitors in *PTEN*-mutant resistant breast cancer cells and xenograft tumors.[55,56]

Compared with the upstream receptors and mediators, the effectors from RTKs signaling pathway are numerous and diverse. Therefore, it is expected that they may be influenced by a number of signaling pathways, and the outcome from the cross-talk involving effectors may be much weaker than the cross-talk at the receptor or mediator level. However, several lines of evidence have demonstrated that an effector controlled by multiple mediators from the same RTK signaling or other pathways causes signal redundancy and attenuates drug sensitivity. For example, inhibition of one mediator can be compensated by another mediator which can activate the same effector. The role of a single downstream effector in the resistance to HER family inhibitors is not well demonstrated in the clinic, but the signaling cross-talk at the effector level may influence the efficacy of the targeted drugs. Furthermore, the combination of inhibitors targeting receptor/mediator has been shown to have high toxicity due to side effects, whereas the combination of inhibitors targeting downstream effectors may limit the toxicity. In the following section, we introduce several key downstream effectors that have a role in TKI sensitivity (Figure 3).

KEY DOWNSTREAM EFFECTORS OF EGFR/HER2 PATHWAYS THAT ARE CRITICAL TO TKI SENSITIVITY

As stated above, studies have shown that inhibition of both RAS/RAF/MEK/ERK and PI3K/AKT/mTOR pathways is sufficient to inhibit proliferations or to induce apoptosis in various RTK-addicted cancer cells.[22,57] Also, reactivation of either RAS or PI3K by overexpression of constitutively active mutants renders cells resistant to RTK inhibition.[57] Because these two pathways can rescue the inhibition of another pathway, it is possible that both pathways regulate multiple common downstream targets. Indeed, there are several common targets shared between these two signaling pathways that are essential for cell survival and proliferation. The most-studied common targets are mTOR and its downstream targets. Both AKT and ERK can activate mTOR by phosphorylating mTORC1 regulators, such as tuberous sclerosis 2 protein (TSC2).[58,59] The main downstream targets of mTOR are S6K1 (p70 ribosomal protein S6 kinase 1) and 4EBP1 (eIF4E binding protein 1).[60] S6K1 is phosphorylated and activated by mTOR to promote translation and growth by phosphorylating cellular substrates, including ribosomal S6, leading to increased ribosomal biogenesis. 4EBP1, which inhibits cap-dependent mRNA translation by binding to the translation initiator eIF4E, is inactivated by mTOR-mediated phosphorylation, resulting in increased cap-dependent protein translation. Thus, mTOR promotes cell growth by promoting protein translation and has an essential role in cancer.[61] It has been shown that 4EBP1 has a key role in oncogenic AKT and ERK signaling in cancer cells with both *PIK3CA* and *RAS* mutations.[62] Moreover, it has recently been shown that the mTOR pathway can activate the Hedgehog (Hh) pathway through S6K1-mediated Gli1 phosphorylation and that mTOR inhibitors (rapamycin, RAD-001)

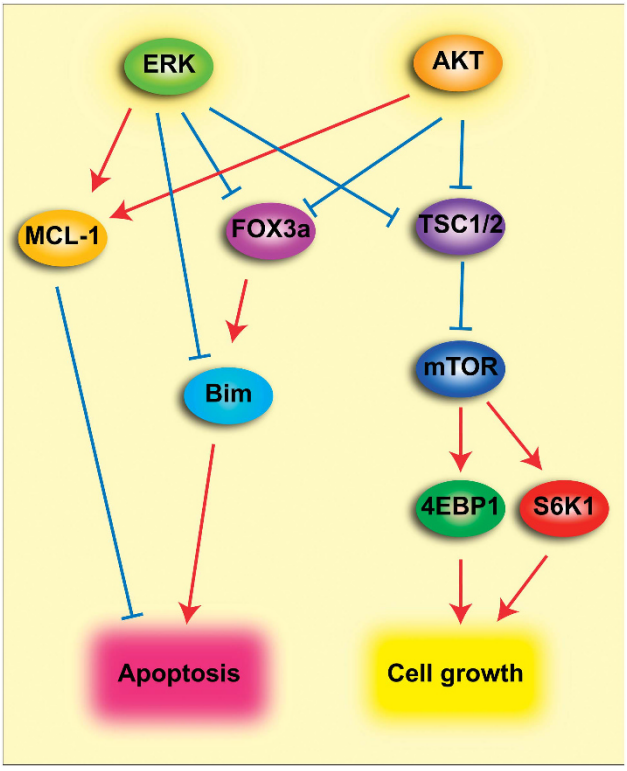

Figure 3. Key effectors for cell survival and growth regulated by both ERK and AKT. TSC and FOXO3 are tumor suppressors that control cell growth and survival, and the activation of these molecules are regulated by both ERK and AKT. TSC regulates mTOR activation, which is critical for cell growth and survival. FOXO3 is a transcription factor that regulates multiple cell cycle and apoptosis regulators, including Bim. Mcl-1 and Bim are anti- and pro-apoptotic Bcl-2 family proteins, respectively. Bim is directly phosphorylated by ERK and undergoes proteasome-dependent degradation. Both AKT and ERK can inhibit GSK3β-mediated Mcl-1 degradation. It has been shown that these molecules have an important role in EGFR/HER2 inhibitor-mediated apoptosis.

can augment the anticancer activity of HH pathway inhibitors (GDC-0449, cyclopamine).[63]

Effectors, including proapoptotic Bcl-2 family protein Bim and antiapoptotic Bcl-2 family protein Mcl-1, are known to have essential roles in apoptosis induced by EGFR/HER2 inhibitors.[22,64–66] Bim mRNA transcription is mainly controlled by the forkhead O transcription factor FOXO3a in growth factor-mediated signaling pathway,[67] and we and others have shown that FOXO3a is a common target of AKT and ERK.[68,69] Bim itself is also directly phosphorylated by both AKT and ERK such that AKT-mediated phosphorylation of Bim attenuates its proapoptotic activitiy, and ERK-mediated phosphorylation of Bim promotes its ubiquitination and degradation.[70,71] Another member of the Bcl-2 family Mcl-1 is phosphorylated by GSK3β, which then undergoes ubiquitination and degradation.[72] Because GSK3β is inactivated by AKT and ERK through phosphorylation,[73,74] both AKT and ERK can inhibit GSK3β-mediated Mcl-1 degradation. We also showed that ERK increases the stability of Mcl-1 via direct phosphorylation.[75] In addition, AKT and ERK have been shown to upregulate Mcl-1 transcription.[76,77] These findings suggest that the expression and activity of Bim and Mcl-1 are strictly controlled by MEK/ERK and PI3K/AKT pathway and that the apoptosis mechanism regulated by Bim and Mcl-1 has a critical role in cell death induced by the inhibition of RTK signaling. Indeed, BH3 mimetic agent ABT-737, which functions in a manner similar to BH3-only proteins and inhibits Bcl-2, effectively induces apoptosis in EGFR TKI-resistant lung cancer cells.[78] In addition to Mcl-1, antiapoptotic Bcl-2 family proteins Bcl-2 and Bcl-XL also have a redundant role in the antiapoptotic mechanism. Therefore, even if Mcl-1 is downregulated by EGFR/HER2 inhibitors, Bcl-2 and/or Bcl-XL regulated by other signaling pathways may contribute to resistance. Both Bcl-2 and Bcl-XL transcription are also regulated by nuclear factor-κB (NF-κB),[79,80] which has an important role in cell survival in inflammation-mediated signaling pathway as we will mention later. Thus, blocking of all three antiapoptotic Bcl-2 family proteins may be an alternative strategy to overcome the resistance. For example, proapoptotic BH3-only protein Bik can block these three antiapoptotic Bcl-2 family proteins,[81] and we showed that mutant Bik (BikDD), which is more potent than wild-type Bik, has an equivalent function of cosilencing three antiapoptotic Bcl-2 family proteins.[82] BikDD has been used as a therapeutic gene in multiple cancer types.[82–85]

In addition to Bim and Mcl-1, p53, a critical tumor suppressor regulating apoptosis and cell cycle in response to various stresses is also regulated by a similar mechanism. MDM2 is the E3 ligase that controls p53 expression via ubiquitination and subsequent proteasome-dependent degradation.[86] It has been shown that MDM2 can be directly phosphorylated by AKT and ERK to increase its nuclear localization and activity.[87,88] Thus, the tumor suppressor role of p53 is likely to be negatively affected by HER family RTK inhibitors. Another unique effector of EGFR/HER2 is STAT3, a transcription factor involved in cell survival and promotes tumor progression.[89,90] STAT3 is directly phosphorylated by Src via EGFR or HER2 activation to promote cell survival[91,92] and is also activated by the IL-6-JAK pathway independently of EGFR/HER2. To date, several studies have suggested that STAT3 activated by JAK is involved in the resistance to EGFR inhibitors.[93–96] Moreover, Src activation has also been implicated in trastuzumab resistance in breast cancer.[97] Therefore, the combined inhibition of EGFR/HER2 and STAT3 or JAK/Src may have the potential to overcome resistance to EGFR inhibitors. Indeed, STAT3 inhibition exhibits synergistic effects in various cancer cells, when combined with EGFR inhibitors.[93,96,98]

Aside from these above-mentioned molecules, there are still numerous common effectors from the RAS/RAF/MEK and PI3K/AKT pathways such as p300, GSK3β and Snail.[99–101] These effectors have essential roles in RTK-mediated cancer cell growth, proliferation, survival and migration/invasion. Their inhibition or activation by signaling pathways other than RAS/RAF/MEK or PI3K/AKT further strengthens the importance of signaling cross-talk in the resistance to EGFR/HER2 inhibitors. In particular, IκB kinase (IKK)α and IKKβ have been demonstrated to share multiple common targets with ERK/AKT,[102] and may function as major survival kinases in the inflammation signals including tumor necrosis factor receptor (TNFR). Thus, we introduce the possible cross-talk of TNFα/IKKα/β with EGFR/HER2 signaling pathways in the next section.

POSSIBLE CONTRIBUTION OF OTHER SIGNALING PATHWAYS IN TKI RESISTANCE AT VARIOUS LEVELS

In this section, we introduce the roles of the Notch, Wnt/β-catenin and TNFα/IKK/NF-κB pathways in cancer (Figure 4), and discuss their potential roles in the resistance to EGFR/HER2 inhibitors via signaling cross-talk at various levels. The Notch signaling pathway is essential for normal development, but dysregulation of this pathway also contributes to tumorigenesis.[103] The Notch receptor family has four highly homologous members, Notch1, Notch2, Notch3 and Notch4. Upon the binding of a Delta or Jagged ligand family member, the Notch receptor is cleaved to release its intracellular domain (Notch intracellular domain), which can then translocate into the nucleus.[104] In the nucleus, Notch intracellular domain interacts with the coactivator to induce the transcription

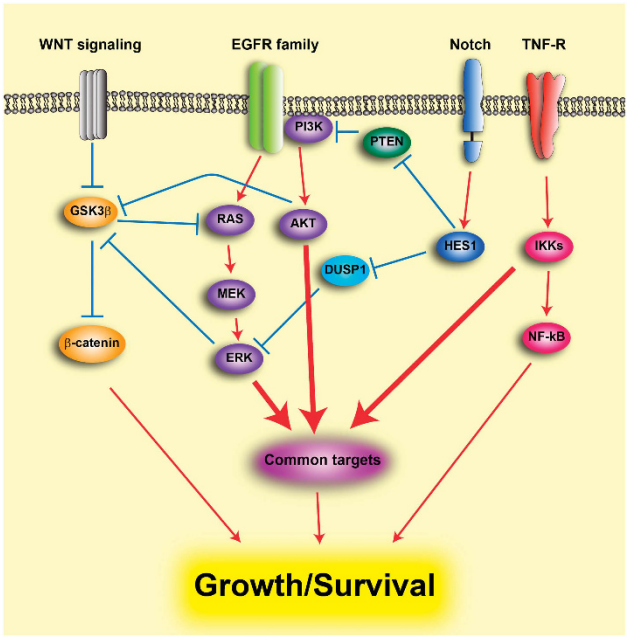

Figure 4. Cross-talk of EGFR/HER2 signaling with other pathways. Wnt/β-catenin, Notch and TNFα/IKK/NF-κB signaling pathway can cross-talk with the EGFR/HER2 pathway at various points and affect the sensitivity to EGFR/HER2 inhibitors. Notch target gene *HES1* encodes a transcription repressor that can inhibit the expression of DUSP1 and PTEN, which are negative regulators for ERK and AKT, respectively. IKK activated by TNFα has several common targets with ERK and AKT, including FOXO3, TSC complex. In the Wnt/β-catenin pathway, GSK3β, a negative regulator of this pathway, is phosphorylated and inactivated by AKT and ERK. GSK3β can phosphorylate and induce β-TrCP-mediated degradation of RAS.

of downstream target genes.[104] Notch has been shown to cross-talk with EGFR/HER2 signaling mainly at the mediator level. For example, activated Notch1 promotes melanoma cell growth and survival via ERK and AKT activation.[105] Notch target gene *HES1* encodes a transcription repressor that can inhibit DUSP1 and PTEN expression.[106,107] DUSP1 is a ubiquitin ligase for ERK, and inhibition of DUSP1 by HES1 activates ERK.[107] Interestingly, EGFR signaling inhibits Notch1 gene transcription[108] whereas HER2 inhibition activates Notch1.[109] Therefore, TKI treatment may activate Notch signaling via a feedback mechanism. Indeed, simultaneous inhibition of Notch and EGFR was reported to induce synthetic lethality in basal-like breast cancer cells.[110] Furthermore, Notch1 activation is associated with EGFR TKI resistance in PC9 cells expressing mutated EGFR.[111] These studies suggest that Notch and RTK signaling pathway may cross-talk at the mediator level and contribute to TKI resistance. Because Notch pathway inhibitors have been tested for cancer treatment, a combination strategy of EGFR/HER2 inhibitors and Notch pathway inhibitors may be effective to overcome the Notch-related resistance.[112]

The canonical and non-canonical Wnt/β-catenin pathways function in both embryonic development and tumorigenesis with the canonical having a more important role in cancer.[113] Wnt ligand binds to Frizzled and LRP5/6 receptor, and initiates a signaling pathway that inhibits GSK-3β-mediated phosphorylation and subsequent ubiqutination- and proteasome-dependent degradation of β-catenin.[114] Stabilized β-catenin translocates to the nucleus where it interacts with TCF/LEF to regulate target gene transcription such as myc and cyclin D.[115,116] The Wnt/β-catenin pathway can also cross-talk with the EGFR/HER2 pathway at various levels. Because this pathway has an important role in colon cancer development, the interplay of the signaling pathways likely exists more commonly in colon cancer cells. GSK3β, a negative regulator of Wnt/β-catenin pathway, can phosphorylate and induce β-transducing repeat-containing protein-(β-TrCP)-mediated degradation of RAS in colon cancer cells.[117] Interestingly, it has recently been shown that β-catenin alters FOXO3a function from proapoptotic to metastasis inducer, and confers resistance to PI3K and AKT inhibitors.[118] In colorectal cancer cells, upregulation of MET at both the mRNA and protein levels by β-catenin contributes to the resistance at the receptor level.[119]

Conversely, RTK signaling can also affect Wnt/β-catenin signaling pathway in multiple ways. One approach by which RTK signaling activates the Wnt/β-catenin pathway is via GSK3β, which is phoshphorylated by AKT and ERK.[73,74] EGFR activation causes the dissociation of β-catenin from α-catenin and activates it via ERK/CK2α-mediated phosphorylation of α-catenin.[120] Moreover, in response to EGFR signaling, pyruvate kinase M2 translocates to the nucleus where it binds to β-catenin and induces β-catenin target genes.[121] In contrast, B-RAF has been shown to inhibit the Wnt/β-catenin pathway to function as proapoptotic regulators when B-RAF is blocked in *B-RAF*-mutated melanoma.[122] Together, the interplay between the Wnt and TKI signaling pathways at various levels impacts the cellular response to EGFR/HER2 inhibitors. However, as the cross-talk between Wnt/β-catenin and EGFR/HER2 may be dependent on cell types, it would be critical to further investigate in *EGFR*-mutant lung cancer cells or HER2-positive breast cancer cells.

The IKK/NF-κB signaling pathway also has an important role in tumor initiation and progression in various cancers.[123] NF-κB is a transcription factor that is critical for cancer cell survival. Several cytokines and inflammatory stimuli such as TNFα, interleukin-1 and lipopolysaccharide can activate this pathway. There are two distinct IKK/NF-κB signaling pathways: canonical and non-canonical. Among the IKK family members, IKKβ is the dominant kinase that regulates NF-κB activity in the canonical pathway whereas IKKα is the dominant kinase in the non-canonical pathway. The detailed mechanisms of IKK-mediated NF-κB activation are discussed in several reviews.[123–125] Indeed, EGFR TKI resistance via NF-κB signaling was identified through a non-biased RNA interference screen in lung cancer cells with mutated *EGFR*.[126] Interestingly, STAT3, which can be activated by EGFR, also promotes nuclear localization and activation of NF-κB via p300-mediated acetylation.[90]

Numerous studies have shown that IKK phosphorylates multiple substrates independently of NF-κB activity.[102] More importantly, these direct IKK substrates include the effectors from the EGFR/HER2 signaling, resulting in the cross-talk between IKK and RTK signaling.[99,127,128] For example, we previously showed that IKKβ phosphorylates and inhibits TSC1, resulting in the activation of mTOR pathway.[129,130] We also demonstrated that IKKβ directly phosphorylates FOXO3a and induces β-TrCP-mediated ubiquitination and degradation.[131,132] Because both FOXO3a and mTOR are key downstream effectors of RTK signaling, IKKβ may impact on the RTK signaling through these interplays. Using a non-biased RNA interference screen, inhibition of IKK was reported to sensitize mutated EGFR lung cancer cells to TKI-induced apoptosis through NF-κB.[126] However, it is not yet clear whether IKKβ-mediated activation of mTOR/S6K1 pathway or inhibition of FOXO3a may contribute to the TKI sensitivity.[129,131] In addition, IKK has been shown to affect the Wnt and Notch signaling pathways such that IKKβ is responsible for expression of the Notch target HES1 in *K-RAS*-mutant pancreatic cancer transgenic mice model.[133] Moreover, IKKα inhibits FOXA2 by phosphorylating it, resulting in the decrease of NUMB expression and subsequent NOTCH activation.[134] IKKα also phosphorylates β-catenin to inhibit its subsequent ubiquitination and degradation.[135] Together, these findings suggest that the IKK/NF-κB signaling pathway has an

important role in the resistance to RTK inhibitors, although it may be in a cell type- or context-dependent manner. With all these cross-talk, further investigation is critical to dissect detailed mechanisms of the cross-talks and will shed light on the development of effective rational combination therapy.

MECHANISMS OF RESISTANCE INDEPENDENT OF SIGNAL CROSS-TALK

About half of TKI resistance mechanisms found in NSCLC patients are caused by the acquired secondary mutation of *EGFR* from threonine 790 to methionine (T790M).[15] This type of EGFR mutant cannot be inhibited by first-generation EGFR TKIs, for example, gefitinib and erlotinib.[136] A second generation of irreversible TKIs, such as afatinib and neratinib, are currently being tested in clinical trials, and the combination of afatinib and cetuximab has demonstrated effectiveness against EGFR T790M mutant.[137] The detailed mechanisms of secondary mutations are introduced in another review.[138] A secondary mutation is also a common mechanism in the resistances to other TKIs, such as those targeting Bcr-Abl and ALK.[139,140] In addition to secondary mutations, NSCLC- and HER2-positive breast cancer cells with epithelial-to-mesenchymal transition phenotype have been shown to be resistant to EGFR TKI and trastuzumab, respectively.[141,142] Interestingly, systematic genetic and histological analyses from drug-resistant NSCLC patient revealed that TKI-sensitive NSCLC could transform into small-cell lung cancer and become resistant to EGFR TKI.[50]

CONCLUDING REMARKS

In this review, we introduced several mechanisms of the resistance to the inhibitors targeting the HER receptor family proteins, including some that are well recognized and some that need to be further validated (Figures 2–4). Moreover, we categorized these cross-talk mechanisms by their actions at different levels of signal transduction and further discussed how signaling cross-talk at various levels may attenuate the effects of EGFR/HER2 inhibitors. Some of the pathways have never been shown in connection with the resistance to EGFR/HER2 inhibitors in the clinic. However, they have the potential to be used as drug targets in combination with EGFR/HER2 inhibitors.

Although we were not able to cover all of the potential signaling pathways that are associated with the resistance to EGFR/HER2 inhibitors, we believe that other signaling pathways such as Hedgehog, Hippo, AMPK/LKB and Aurora A/B may also contribute to the resistance via their cross-talk with the EGFR/HER2 signaling pathway at various levels. Because the association of each signaling pathway with others at various levels complicates the cross-talk, more mechanistic studies will be needed to further dissect its impact. In addition, identification of a key signal node triggering the cross-talk between different pathways will be an important area to explore. For instance, the signal node may be a protein complex such that its inhibition could be the key to blocking cancer cell growth and inducing cancer cell death.

Finally, miRNA has recently been recognized as an important mediator of signal transduction via regulation of mRNA stability and translation. In fact, some miRNAs have been suggested to function in EGFR TKI resistance in lung cancer.[143] It would be of importance to also explore this emerging area of research to further understand how miRNAs contribute to signaling cross-talk that leads to drug resistance. As extensive studies in signal transduction have successfully led to the development of current targeting agents, we believe that more effective and alternative strategies will soon emerge to overcome signaling cross-talk-mediated drug resistance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported in part by the National Breast Cancer Foundation, Sister Institution Fund of China Medical University Hospital and MD Anderson Cancer Center, Patel Memorial Breast Cancer Research Fund, Center for Biological Pathways, and CCSG (CA16672).

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