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The HER family and cancer: emerging molecular mechanisms and therapeutic targets

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Abstract

The human epidermal growth factor receptor (HER) family of transmembrane tyrosine kinases regulate diverse cellular functions in response to extracellular ligands. The deregulation of HER signaling through gene amplification or mutation is seen in many human tumors and an abundance of experimental evidence supports the etiologic role of these events in cancer pathogenesis. In addition, the fact that they are feasible targets for both antibody and small molecule therapeutics has made them highly pursued targets for the development of rationally designed anti-cancer drugs. Several HER-targeting agents have entered clinical practice and this has led to novel discoveries regarding mechanisms of resistance, defining a new generation of challenges for targeted cancer therapies. Here we review recent advances in our understanding of HER signaling and targeting in cancer.

Keywords

EGFR; HER2; HER3; ErbB; epidermal growth factor receptor

The HER family

The Human Epidermal Growth Factor Receptor (HER) family is part of the receptor tyrosine kinome and consist of four members; EGFR/ErbB1, HER2/ErbB2, HER3/ErbB3 and HER4/ErbB4. The shared general structure of HER proteins consists of an extracellular domain (ECD), a single span transmembrane (TM) domain and an intracellular domain (ICD) containing the conserved catalytic kinase domain and carboxy terminal tail. A fundamental aspect of signaling in this family is the interaction of two receptors. It is the dimerization of two HER family members and transphosphorylation of their intracellular regions that generates the initial signal leading to activation of numerous downstream signaling pathways. This dimerization event is regulated by extracellular ligands of the epidermal growth factor and neuregulin families. Our understanding of the mechanistic basis underlying receptor interaction and signal generation in this family has been greatly enhanced by crystal structure reports of several soluble HER ECDs in recent years. A key mediator of receptor interaction is a dimerization domain in the ECD (refer to figure 1). In the absence of ligand, this dimerization domain is engaged in an intramolecular interaction and not available for inter-receptor interactions. Ligands bind two non-contiguous areas within the ECD, changing the conformation of the ECD and thereby exposing the

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dimerization interface and making it available for receptor-receptor interactions (recently reviewed in ¹). In parallel to the ECD interactions via their dimerization domains, their intracellular tyrosine kinase domains also interact leading to transphosphorylation of their intracellular regions. The ECD of HER2 is unique in that it is constitutively locked in a conformation resembling the ligand-bound states of the other HER ECDs ^{2, 3}. As such, HER2 is not subject to regulation by ligands and has no ligand, and HER2 is always poised for dimerization with available activated HER members. Dimerization is likely not entirely driven by ECD interactions, and HER protein TM domains are also capable of inducing dimerization ^{4, 5}. But little is known about the structural basis that defines the functional relationships between the ECD, the TM, and the ICD interactions.

The structural intricacies of the kinase domain interactions are less understood, but are beginning to be elucidated. The bi-lobed kinase domain of the EGFR appears to be intrinsically autoinhibited and its activation is felt to be mediated through an asymmetric intermolecular interaction with the C-lobe of the interacting kinase. The HER3 kinase domain is unique in the HER family in that it is catalytically inactive. However, HER3 is an efficient dimerization partner for other HER members and according to this model, it is predicted and expected that the HER3 kinase domain is competent and perhaps efficient at playing the activating role in a kinase dimer. Sequence analysis is consistent with this prediction and indicates that critical residues underlying the kinase pair interactions are invariant across the HER family ⁶.

The multiplication of the HER family to four members in mammals compared with a single member in nematodes has been associated with functional interdependency rather than redundancy. This is exemplified by HER2 and HER3 which are functionally incomplete transmembrane receptors, one lacking binding and regulation by ligands, and the other lacking catalytic kinase function, thereby necessitating interdependency. In fact each of the individual HER genes is essential in development in contrast to other tyrosine kinase families which are essential as a family, but characterized by redundancy among the individual members.

Signaling pathways downstream of HER proteins

Jointly the HER family controls a comprehensive network of survival, growth, metabolism and motility responses by coupling to PI3K/Akt, Raf/MEK/MAPK, JAK/STAT, Src, PLC γ and other pathways (recently reviewed in ⁷). The signaling potency and the selectivity of engagement of these pathways is proscribed by the dimer pair. A hierarchical relationship governs the signaling potency of HER dimers with heterodimers being more active than homodimers, HER2-containing heterodimers being particularly active, and the HER2-HER3 heterodimer being the most active ⁸. Selectivity in engaging downstream pathways is governed by the phosphorylation-dependent and independent consensus binding sequences on each member and have been fairly extensively characterized ^{9, 10}. Uniqueness in the interactomes of the individual HER members adds functional relevance to partner selection in dimerization. Specifically, EGFR and HER4 have much greater diversity in their interacting proteins compared with HER2 and HER3. In fact, HER2 appears to have the fewest interacting proteins consistent with its role as a kinase engine. HER3 is characterized by a large number of binding sites for the p85 regulatory subunit of PI3K. Each of the members has binding sites for the adaptor Grb2 and can potentially activate the Ras/MAPK pathway.

HER activation in cancer

HER family signaling is deregulated in a number of subtypes of human cancers. This has been most commonly recognized and etiologically established in breast cancers, lung

cancers, and glioblastomas (refer to Table 1). Many other cancers have increased expression of HER family members, however their role in other cancers is less well established.

Overexpression of HER2 due to gene amplification is found in 25–30% breast cancer and confers a particularly aggressive biology¹¹. HER2 overexpression is also uncommonly seen in cancers of the esophagus, stomach, ovaries, and endometrium. Mutations in HER2 are rarely found in these cancers and overexpression appears to be the principal mechanism by which HER2 mediates tumorigenesis in these cancers. Numerous experimental models have confirmed that HER2 is potently transforming when overexpressed and several mouse transgenic models have confirmed the role of its rodent homologue *Neu* in mammary tumorigenesis¹². Tumors arising in *Neu* transgenic mice have increased expression of the mouse HER3 homologue, activation of src and activation of the PI3K/Akt signaling pathway (recently reviewed in¹³). Importantly, tumors arising in *Neu* transgenic mice are highly dependent on continued overexpression of *Neu* and in models wherein transgenic expression can be turned off, the disease completely regresses including both the primary mammary tumors and their lung metastases¹⁴. HER2 kinase domain mutations are seen in a small subset of lung cancers, particularly in asian populations¹⁵. These HER2 mutations are associated with increased kinase activity and transformation *in vitro*¹⁶. Transgenic models of these HER2 mutations have not yet been reported.

EGFR is frequently amplified and overexpressed in nearly half of all glioblastomas. In these cancers, amplification is frequently associated with deletion mutations involving the ECD of EGFR of which one particular variant, called EGFRvIII is the most common¹⁷. These mutations result in the constitutive activation of EGFR and are frequently associated with additional mutations in the cell cycle regulatory gene INK4a-ARF. Mouse transgenic models confirm that mutant EGFR is tumorigenic, but requires additional mutations in cell cycle arrest pathways¹⁸.

EGFR is altered through point mutation or deletion mutation in the kinase domain in 10–15% of NSCLCs in the U.S. and in 30–50% of NSCLCs in Asia. The mutations are clustered within four exons encoding the kinase domain^{19–22}. These mutations produce a spectrum of biochemical effects from increased kinase activity to ligand-independent constitutive activity^{21, 23, 24}. Destabilization of the autoinhibited conformation of the EGFR ICD has been proposed to explain constitutive activation of these EGFR mutants²³. Based on the solved EGFR ICD structure, the L858R mutation in the A-loop and the deletion Del(746–750) in the α -C helix are predicted to disrupt the autoinhibited ICD. EGFR is overexpressed without mutation in a much larger subset of NSCLCs, however its etiologic role in these scenarios is not well established. The etiologic role of at least some of the common EGFR mutant alleles in tumorigenesis has been confirmed by *in vitro* and *in vivo* experiments. These EGFR mutants are transforming in fibroblast and epithelial cell models, and cause lung tumors when expressed in mice lung epithelia^{25–28}. EGFR is also widely overexpressed in many other epithelial cancers including cancers of the head and neck, ovary, cervix, bladder, esophagus, stomach, endometrium, colon and breast.

HER3 is not currently characterized as a proto-oncogene and significant genomic level alterations in HER3 have not been found in tumors²⁹. However this does not undermine its role and its activation in cancers driven by EGFR or HER2. A significant body of data suggest that HER2 and HER3 are partners in signaling and in transformation. The HER2-HER3 heterodimer is the most active signaling dimer within the HER family⁸. Tumors that arise in mice due to oncogenic ErbB2 have increased expression of ErbB3 and similarly, tumors from patients with HER2 amplified breast cancer have increased expression of HER3³⁰. HER3 is an obligate partner for HER2 in transformation and HER2 is unable to transform cells in the absence of HER3³¹. HER3 is likely also involved in EGFR-driven

tumors, although this relationship is not as well characterized. The mutational activation of EGFR in lung cancers is associated with the phosphorylation of HER3 and coupling of HER3 to PI3K ³².

HER4 is the least well characterized member of the HER family. But existing evidence does not implicate HER4 overactivity in tumorigenesis, and in fact HER4 signaling has been associated with differentiation, cell death, or reduced tumorigenicity ^{33, 34}. Very rare mutations of the HER4 kinase domain have been reported in one study, but at this time the biological significance of this finding is not clear ³⁵.

Although many questions still remain (Text box 1) the evidence to date clearly implicates HER proteins in the pathogenesis of certain subtypes of human cancer.

Text Box 1

Outstanding questions in HER biology

- How does dimerization induce activation of the kinase domain?
- Do ligands play a role in the progression of cancers with HER mutation or amplification?
- Are cancers without HER mutation or alteration dependent on HER signaling?
- Does HER4 have a role in tumorigenesis?
- Why are EGFR mutations in lung cancer so much more common in Asia?

Effectors of oncogenic HER signaling

The frequent activation of HER proteins in many types of human cancer attest to their critical role in regulating pathways important for tumorigenesis and tumorigenic survival. These pathways include the Ras-MAPK pathway, the PI3K/Akt pathway, the JAK/Stat signaling pathway, and PLC γ . The significance of these pathways in tumorigenesis is underscored by the fact that the activation of each of them is frequently found in many types of cancers. However there are both similarities and differences in the abilities of EGFR or HER2 to influence these pathways. Activated EGFR and HER2 can both increase Ras-MAPK signaling making additional mutations in this downstream pathway redundant. Consistent with this notion, Ras and B-Raf mutations although common across all cancers, are rare in EGFR-mutated lung cancers, in HER2 overexpressing breast cancers, or in EGFR amplified glioblastomas ^{36–38}. STAT3 is activated by EGFR and HER2 and is activated in EGFR mutated lung cancers and glioblastomas ^{39–42}.

The PI3K/Akt pathway appears to be a pivotal pathway for tumorigenesis and is widely activated in many tumors including tumors driven by activated EGFR or HER2. EGFR and HER2 do not have direct binding sites for PI3K. However HER3 has seven PI3K-binding sites and when phosphorylated by EGFR or HER2, is a potent activator of PI3K ⁴³. EGFR can also activate PI3K through the docking protein Gab1 ⁴⁴. HER2 amplified breast cancers have increased expression and phosphorylation of HER3 and increased activity of downstream PI3K/Akt signaling ³⁰. Akt can also be activated through the loss of PTEN in many cancers, however loss of PTEN is rarely seen in HER2 amplified breast cancers, consistent with its presumed redundancy when HER2-HER3 signaling is activated ⁴⁵. Activating mutations of PI3K are however seen in HER2 amplified breast cancers. Since HER2 is capable of activating PI3K signaling through its partner HER3, it is currently difficult to hypothesize the advantage conferred by PI3K mutation in HER2 amplified tumors. It is possible that some of the functions of mutant PI3K are not redundant with the

upstream activation of wildtype PI3K by HER3. For example, mutationally activated PI3K is active within the cytoplasmic compartment, whereas HER2-HER3 activated PI3K is active primarily at the plasma membrane.

EGFR-mutant lung cancers also have activation of HER3 and downstream PI3K/Akt pathway, and these are not known to be associated with mutations in PI3K or PTEN^{32, 46}. However EGFR amplified glioblastomas have frequent activation of Akt and in these cancers this is often due to the loss of PTEN. It is currently difficult to understand the advantage conferred by the loss of PTEN in EGFR amplified glioblastomas, since it would seem to be redundant if activated EGFR can signal the activation of HER3 and PI3K/Akt signalling. However there is a lot more that is to be learned about EGFR signaling in glioblastomas and compared with EGFR signaling in lung cancers. It is possible that the EGFR oncogenes with ECD mutations commonly seen in glioblastomas are not intrinsically efficient at activating HER3, necessitating PTEN deletion to activate Akt signaling. On the other hand, the EGFR kinase domain mutations in lung cancer increase the ability of EGFR to activate the PI3K/Akt pathway, and both HER3 and Gab1 have been implicated in this circuitry^{32, 46}. Clearly much more work in this area is needed to better understand the differences between activated EGFR in lung cancers and in glioblastomas.

Targeting HER proteins in the treatment of cancer

The abundance of evidence that implicates overactive HER signaling in the genesis and progression of a number of human cancers has led to the development of drugs that selectively target these proteins. Two classes of drugs have thus far have shown clinical efficacy and much of the ongoing pharmaceutical efforts continue to be in the realm of these two classes. One is monoclonal antibodies that can target extracellular epitopes and the other is small molecule cell permeable inhibitors of catalytic kinase function. The agents that have been approved by the FDA for clinical use are listed in Table 2.

Monoclonal antibodies

Monoclonal antibody (mAb) therapies are highly selective agents that can bind with one but not other members of the HER family. They have wide therapeutic indices and are generally not given at maximum tolerated doses, since they are not felt to have dose response effects above serum concentrations that saturate their targets. In addition to their potential to interfere with the signaling functions of their targets, mAbs, particularly of the IgG1 class, have the ability to activate antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC), and when targeted to antigens overexpressed in tumors, these activities can comprise a particularly significant component of their anti-tumor activities. The technologies to create humanized antibodies has eliminated problems associated with the development of anti-mouse antibodies in patients. The earliest hypothesis driving the development of mAbs against the HER ECDs was that they would interfere with ligand binding and activation of HER proteins. This however presumes that cancer progression requires ligand activation, and this is a hypothesis that remains to be proven. There are currently no experimental models that can effectively study the role of ligand expression by host tissues. However experimental models reveal that some EGFR-expressing cancer cell lines secrete EGFR ligands themselves creating autocrine loops to drive their proliferative activities and mAbs can interrupt such autocrine signaling loops⁴⁷.

Cetuximab was the first such anti-EGFR mAb developed for clinical use. This IgG1 class mAb has little activity against EGFR overexpressing breast cancers but has clinical activity in the treatment of head and neck cancers which frequently express EGFR⁴⁸. Cetuximab also shows activity against colon cancers but this activity has no correlation to tumor expression of EGFR and in fact cetuximab also shows activity in colon cancers without

measurable EGFR expression on immunohistochemistry^{49, 50}. It remains possible that lower levels of EGFR undetectable by immunohistochemistry are nevertheless functionally important. Because of its proven clinical activity, cetuximab is now used commonly in combination with chemotherapy and radiotherapy in the management of some cancers. However the mechanism of action of cetuximab and the tumor molecular characteristics that predict response to it remain to be defined. Panitumumab is a newer anti-EGFR mAb, of the IgG2 class, and has similarly shown evidence of modest clinical activity in patients with colon cancer with no apparent correlation with tumor EGFR expression⁵¹. Therefore although two anti-EGFR mAbs show clinical activity and have successfully entered into clinical practice, there is currently no compelling mechanistic model for their clinical activities that explains why some tumors are responsive and others resistant.

The anti-HER2 mAb trastuzumab was developed with a similar hypothesis in mind regarding inhibiting ligand-activation of HER2. Trastuzumab indeed shows clinical activity in breast cancers, and in contrast with the EGFR-targeting mAbs, trastuzumab is only active against cancers that overexpress its target HER2^{52, 53}. However evidence emerging after the development of trastuzumab revealed that HER2 has no ligand and is not subject to ligand activation. Numerous alternative hypotheses to explain the mechanism of activity of trastuzumab have been proposed, including inhibition of HER2 association with other membrane receptors, however none of these have been confirmed and the mechanism of activity of trastuzumab remains unknown (reviewed in⁵⁴). Trastuzumab is however an IgG1 class mAb with significant ADCC activating function and a significant body of evidence implicates an immunologic mechanism of action for trastuzumab⁵⁵.

Since the development of trastuzumab, our understanding of the structural basis underlying the activation of HER signaling has significantly increased and current evidence allows targeting HER2 epitopes with higher potential for functional interference. The anti-HER2 mAb pertuzumab is such a second generation agent. Pertuzumab targets the dimerization interface of HER2 and potentially interferes with HER2 dimerization including HER2-HER3 dimerization^{56, 57}. Clinical testing of pertuzumab is currently ongoing, and preliminary evidence shows promising activity against HER2 overexpressing breast cancers⁵⁸.

Tyrosine kinase inhibitors

Improvements in potency and selectivity of tyrosine kinase inhibitors (TKIs) led to the development of clinically useful agents in the late 1990s based on the quinazoline structure and the repertoire of agents has rapidly expanded such that there are now numerous agents in preclinical and clinical stages of development. Three HER-targeting TKIs have thus far been approved for clinical use. These include gefitinib (Iressa®), erlotinib (Tarceva®), and lapatinib (Tykerb®). TKIs do not have the singular specificities of mAbs and they invariably have activity against multiple members of the HER family. Gefitinib and erlotinib are most selective for EGFR *in vitro*, while lapatinib targets both EGFR and HER2 equally *in vitro*. Their selectivities within the HER family are less apparent in cell based systems where inactivation of HER family signaling occurs even with the most selective agent⁵⁹. In addition, many TKIs have numerous off-targets outside of the HER family which can potentially increase their toxicities and limit their therapeutic indices⁶⁰. Although TKIs don't match the selectivities of mAbs, they have a more solid mechanistic basis since they inhibit catalytic function of HER proteins which is essential for oncogenic function, and their mechanism of action does not presume a requirement for ligand binding or other extracellular interactions. In addition, since they are competitive enzymatic inhibitors with dose-response effects and are potentially more potent agents compared with mAbs.

The clinical testing of HER family TKIs was initially undertaken in lung cancers based on the hypothesis that HER signaling is broadly important in lung cancers. However the finding of clinical activity in some NSCLCs led to the discovery of EGFR kinase domain mutations in a subset of these cancers²⁰. Further clinical studies have now established that the HER TKIs gefitinib and erlotinib have clinical anti-tumor activity in about 70–80% of patients with mutant EGFR-driven lung cancers (reviewed in⁶¹). Tumors driven by mutant EGFRs can be highly sensitive to TKIs. The exquisite sensitivity of these tumors appears to be due to structural changes incurred by the kinase domain mutation that renders them significantly more sensitive to TKIs compared with wildtype EGFR²⁴.

Although tumor EGFR mutation is a highly predictive marker of lung cancer response to HER TKI therapy, the clinical activities of TKIs do not seem to be limited to cancers with EGFR mutation. Some lung cancers with wildtype EGFR are also responsive to HER TKI therapy⁶². Identification of molecular characteristics that can predict response to TKI therapy in patients with wildtype EGFR lung cancers is the subject of numerous ongoing studies and there is no clear consensus about this yet. There are conflicting reports regarding whether the level of EGFR protein expression predicts response to TKI therapy^{63, 64}. This may be partly due to the technical limitations of assaying protein expression by immunohistochemistry. Assaying DNA targets is somewhat more objective and less subject to artifacts, and some investigators have proposed that increased EGFR gene copy number, either through gene amplification or polysomy, may be a predictive marker of response^{63, 65}. However EGFR amplifications are frequently associated with EGFR mutations and whether amplified but wildtype EGFR containing tumors are sensitive to TKI therapy remains to be defined. Other reports also suggest increased HER2 gene copy number or HER2 kinase domain mutation may predict response to TKI therapy^{66, 67}. In contrast to these clinical studies, preclinical models do not show a relationship between EGFR/HER2 expression and response to TKI therapy^{64, 68}. The effort to identify the optimal predictive molecular markers of response to TKI in lung cancers continues in the academic arena, and the use of molecular biomarkers has not yet been incorporated into standard clinical practices.

HER TKIs are promising agents for the treatment of breast cancers driven by EGFR or HER2 overexpression and these tumors constitute 25–30% of all breast cancers. However the promise of this treatment hypothesis has not yet been realized. Several clinical studies of the HER TKIs gefitinib, erlotinib, and lapatinib show only limited efficacy in breast cancers, even in selected patients with HER2 or EGFR overexpressing tumors^{69–72}. This is despite an abundance of experimental evidence suggesting that HER2 overexpressing tumors are driven by HER2 and highly dependent on HER2 activity. The reasons for this are not entirely clear but recent evidence discussed below suggests that this may be due to ineffective suppression of HER2-HER3 transphosphorylation. Efforts to increase the activities of HER-targeted therapies for the treatment of HER2 overexpressing breast cancers have included combinations of mAbs and TKIs. The combination of gefitinib and trastuzumab showed little evidence of clinical activity and combination studies of lapatinib and trastuzumab are ongoing⁷³. Effective treatment of HER2 overexpressing breast cancers with TKIs continues to be a matter of investigation and the current mechanistic evidence discussed below seems to suggest that HER2 appears to be a more resilient target for TKIs than EGFR, and the successful treatment of these cancers may have to await more effective inhibitors of HER2 signaling.

Since the mutational activation and amplification of EGFR is frequently seen in glioblastomas, TKIs are promising agents for the treatment of this disease. However there are specific challenges in the pharmacologic therapy of glioblastomas including the poor distribution of drugs within the brain parenchyma due to the blood-brain barrier. Small

molecule TKIs have a much better chance of crossing the blood-brain barrier than mAbs, specially in patients with brain tumors where there may be local disruption of the barrier by the tumor. Indeed gefitinib and erlotinib have modest activity in patients with glioblastoma^{74, 75}. Studies in this disease are ongoing using higher doses of TKIs and combination therapies with TKIs.

Mechanisms of resistance to HER TKIs

There is now a considerable amount of experience in the study of denovo and acquired TKI resistance and in vitro and clinical studies have begun to elucidate the molecular mechanisms by which tumors can evade TKI therapy. In patients with EGFR-mutant lung cancers, it is now clear that resistance to TKI therapy invariably develops^{76, 77}. More than half of these are the T790M mutation within the kinase domain which blocks binding of erlotinib or gefitinib⁷⁷. These mutations are similar to acquired mutations that develop in the Bcr-abl and kit oncoproteins exposed to TKIs⁷⁶. Efforts are ongoing to identify structurally distinct TKIs that overcome the T790M mutation. This effort appears hopeful since structures have already been identified which appear to be effective against mutations at this residue⁷⁸. Acquired resistance to TKI therapy also occurs in the absence of secondary mutations, and the mechanisms underlying this are being intensely pursued. The evidence seems to suggest that TKI resistance is associated with persistent HER3 signaling, and a subset of these appear to be due to amplification of the tyrosine kinase c-MET^{32, 79}. Future development of combination or sequential TKI therapies may ultimately prove much more effective and successful at circumventing drug resistance.

In contrast to EGFR-mutant lung cancers which are initially responsive to TKI therapy but develop resistance after prolonged therapy, HER2 amplified breast cancers appear to be largely resistant to HER-targeted TKIs. The reasons for this are not entirely understood. Correlative scientific studies and tissue pharmacodynamic studies have attempted to define the effects of HER TKIs on their molecular targets and pathways. These studies appear to show that TKIs do reduce EGFR and HER2 autophosphorylation and downstream MAPK signaling, however a reduction in Akt signaling has not been seen^{80, 81}. Tissue biodistribution does not seem to be a limitation and at least in the case of gefitinib, drug levels in tumor tissue appear to be well within the expected therapeutic range⁸². Our laboratory recently gained considerable insight into breast cancer resistance when we found that HER3 escapes inhibition by TKI therapy in HER2-overexpressing breast cancers⁸³. This is due to Akt-driven feedback signaling that reestablishes HER3 signaling by inducing a forward-shift in HER3 phosphorylation-dephosphorylation steady state equilibrium. This work along with previous work showing the obligatory role of HER3 in HER2-induced tumorigenesis reveals that the HER2-HER3 signaling complex represents the principal oncogenic unit in HER2-amplified breast cancers, and effective treatment of this cancer subtype requires the effective inactivation of this oncoprotein complex. The separation of kinase and signaling functions into two proteins in this multi-member family affords a signal buffering capacity inherent in HER3 that makes it considerably more difficult to target this oncoprotein complex in comparison to more simple oncoproteins like Bcr-abl. Efforts are now ongoing to develop more potent TKIs and combinations of TKIs with inhibitors of HER3 effectors such as PI3K inhibitors or Akt inhibitors in order to effectively silence oncogenic HER2-HER3 signaling.

Conclusion

It is now clear that the HER family has significant oncogenic potential that is unleashed in a number of common human cancers. A substantial body of scientific evidence has now provided significant insight into the structural and signaling mechanisms that underly the oncogenic activities of this family. These include overactivity through overexpression,

through gene amplification, through large deletion mutations in their extracellular regions, and through small or point mutations in the kinase domains. Some tumors without detectable structural alterations of the HER family genes and gene products may also be functionally dependent on this family, although such tumors are considerably more difficult to identify due to the lack of identifiable sequence or structural abnormalities in the HER family.

The critical mass of scientific knowledge about HER proteins has made them ideal targets for the development of rationally designed anticancer drugs and one of the most active areas in pharmaceutical sector. Both monoclonal antibodies and small molecule TKIs that target this family of proteins have shown efficacy and are approved for clinical use in a variety of cancer subtypes. Some of these agents have predictive biomarkers that identify patients that are likely to benefit. However there remains clinical activity that is yet unexplained by predictive biomarkers, particularly in cancers without identifiable structural abnormalities of HER family genes, and much work remains to be done to determine predictive tools and assays that can define the full spectrum of activity of these agents.

The success of the first decade of HER-targeting agents has identified a new generation of questions and challenges to be met in the coming decade (Text box 2). At the forefront of these challenges is the quest to overcome resistance to HER-targeting therapies. The coming years will see newer generations of HER targeted therapies come into clinical testing with renewed hope that cancers driven by HER oncogenes can eventually be eradicated using rationally designed therapeutics.

Text Box 2

Current challenges in HER targeting therapeutics

- How can the HER2-HER3 oncoprotein complex in HER2-amplified breast cancers be fully inactivated?
- How can HER3 be inhibited, since it is kinase-inactive?
- How can drug-resistant mutants of EGFR in lung cancer be inhibited?
- How can TKI resistance in PTEN-null glioblastomas be overcome?
- How does trastuzumab work in breast cancers?
- How does cetuximab work in colon cancers?

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Figure 1. The structural basis for the activation of HER proteins

The extracellular regions of HER proteins are composed of four domains numbered I,II,III, and IV. Domains I and III are ligand binding domains, and domains II and IV are cysteine-rich domains which bind each other and other receptors. The tyrosine kinase domain has two lobes which are shared among the tyrosine kinase family and are termed the n-terminal and c-terminal lobes. The c-terminal tail contains many tyrosines which are targets for phosphorylation. When unliganded, the ECD is folded into a closed conformation due to an intramolecular interaction between domains II and IV (see inactive monomer). The HER ligand bind domains I and III bringing them together. The result is a refolding that exposes domain II which is the dimerization interface (see active monomer). The dimerization domains of two active receptors interact, bring the intracellular domains in close proximity and the interaction of the two kinase domains results in transphosphorylation (see dimer).

Table 1

Genomic level abnormalities in HER genes reported in human cancers

Gene	Disease	Alteration	Frequency
EGFR	breast & other cancers	gene amplification	5% in breast cancer, uncommonly in others
	glioblastoma	ECD deletion mutations & gene amplification	40–50%
	non-small cell lung cancer	kinase domain mutation	10% in US/Europe, 30–50% in East Asia
HER2	breast cancer	gene amplification	25–30%
	non-small cell lung cancer	kinase domain mutation	4%
HER3		no significant alterations reported	
HER4	multiple types	kinase domain mutation	1–2% in East Asia

Table 2

HER targeting agents approved by the FDA for the treatment of cancer

Drug	class	molecular target	diseases
cetuximab (Erbix)	IgG1 mAb	EGFR ECD	colon ca, head & neck ca
panitumumab (Vectibix)	IgG2 mAb	EGFR ECD	colon ca
trastuzumab (Herceptin)	IgG1 mAb	HER2 ECD	breast ca
gefitinib (Iressa)*	quinazoline	HER kinase (EGFR>HER2)	lung ca
erlotinib (Tarceva)	quinazoline	HER kinase (EGFR>HER2)	lung ca, pancreatic ca
lapatinib (Tykerb)	quinazoline	HER kinase (EGFR & HER2)	breast ca

* not currently marketed in the US