The basic biology of HER2

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Summary

Human epidermal growth factor receptors (HER/erbB) constitute a family of four cell surface receptors involved in transmission of signals controlling normal cell growth and differentiation. A range of growth factors serve as ligands, but none is specific for the HER2 receptor. HER receptors exist as both monomers and dimers, either homo- or heterodimers. Ligand binding to HER1, HER3 or HER4 induces rapid receptor dimerization, with a marked preference for HER2 as a dimer partner. Moreover, HER2-containing heterodimers generate intracellular signals that are significantly stronger than signals emanating from other HER combinations. In normal cells, few HER2 molecules exist at the cell surface, so few heterodimers are formed and growth signals are relatively weak and controllable. When HER2 is overexpressed multiple HER2 heterodimers are formed and cell signaling is stronger, resulting in enhanced responsiveness to growth factors and malignant growth. This explains why HER2 overexpression is an indicator of poor prognosis in breast tumors and may be predictive of response to treatment. HER2 is a highly specific and promising target for new breast cancer treatments. The recombinant human anti-HER2 monoclonal antibody (rhuMAb-HER2, trastuzumab, Herceptin) induces rapid removal of HER2 from the cell surface, thereby reducing its availability to heterodimers and reducing oncogenicity.

Key words: breast cancer, HER2 (erbB-2, neu), network, signal transduction, tumor growth, tyrosine phosphorylation

Introduction

The neu gene was originally identified in rat neuroectodermal tumors [1] and later its close human relative was isolated. This human gene encodes a protein that has a structure consistent with a growth factor receptor and has been designated HER2, due to its similarity to the human epidermal growth factor receptor [2, 3]. The HER2 receptor has an important role in normal cell growth and differentiation. However, amplification of the HER2 gene leads to overexpression of the receptor, which is linked to the development of many types of human cancers including breast, ovarian and those of the gastrointestinal tract [4, 5].

Breast cancer is a leading cause of cancer-related death in women. In many cases, the cancer is resistant to conventional drugs, resulting in treatment failure. The prognosis for patients with breast cancer is determined by well-recognized pathologic features associated with aggressiveness, histologic grade, tumor size and nodal involvement. Approximately 20%-30% of breast cancers overexpress the HER2 receptor, and it is now recognized that high levels of HER2-receptor expression are an indicator of poor prognosis in patients with breast cancer. Furthermore, overexpression of HER2 is associated with aggressive tumor growth and metastatic activity [4]. Slamon et al. [6] first observed that HER2-gene amplification independently predicted overall (OS) and disease-free survival (DFS) and these findings have been confirmed by subsequent studies [7]. These patients have poor response rates and short DFS and OS compared with women whose tumors do not overexpress this receptor.

There have been attempts to use HER2 status as a predictive factor for response to hormonal and chemotherapy in breast cancer. Although the presence of estrogen receptors indicates response to hormonal therapy, HER2 overexpression is associated with poor response to tamoxifen, possibly because of different growth-signaling pathways. In contrast, HER2 overexpression may be associated with a better response to chemotherapy, particularly anthracyclines, than expected. Therefore, HER2 status is now a well-characterized indicator of aggressiveness and poor prognosis. Moreover, HER2 is being investigated as a target for new breast cancer treatments. Monoclonal antibodies directed against the HER2 receptor are currently the most promising treatments. It is important to understand the biology of the receptor in order to elucidate the mechanism of action of these newly-developed treatments.

The HER family of receptors

The HER/erbB signaling network has developed in parallel with the evolution of complex life forms. The ancestors of HER receptors may be seen in invertebrate organisms such as Caenorhabditis elegans (C. elegans).
and Drosophila melanogaster. These organisms only have one HER receptor. Although the receptor in C. elegans has one ligand, the receptor in fruit flies (Drosophila melanogaster) has four ligands. In contrast, the mammalian HER receptor family comprises four closely-related growth factor receptors that show a high degree of homology with each other. The receptors are all located on the cell membrane and are found in a variety of tissues. They interact with a range of ligands, all sharing an EGF-like motif of 50–55 amino acids, including six highly conserved cysteine residues [8].

The HER2 receptor is encoded by the HER2 gene, a proto-oncogene mapped to chromosome 17q21 [2]. The HER2 receptor is a 1255 amino acid, 185kD transmembrane glycoprotein also designated as p185HER [2]. All four HER receptors comprise a cysteine-rich extracellular ligand binding site, a transmembrane lipophilic segment, and an intracellular domain with tyrosine kinase catalytic activity (Figure 1) [9]. A terminal carboxyl autophosphorylation segment is most likely to be responsible for translation of the activation signals initiated by extracellular ligand binding into physiologic action.

Ligands of HER receptors

An understanding of the action of ligands of HER receptors is crucial to understanding the role of the receptors themselves. The HER receptors exist as monomers. However, on ligand binding they form receptor dimers, which can either be homodimers with the same receptor type (e.g., HER1–HER1) or heterodimers with different receptor types (e.g., HER1–HER2)[10]. Dimer formation is driven by the higher stability of the complex formed between a ligand and two receptors compared with the monomeric receptor.

The HER1 receptor has many roles and is activated by six ligands: epidermal growth factor (EGF), transforming growth factor α (TGFα), amphiregulin, heparin-binding EGF-like growth factor, betacellulin and epiregulin. HER3 and HER4 receptors bind neuregulins (NGFs), a family of structurally diverse peptides [10]. Signaling by all neuregulins is directed through mitogen-activated protein kinase (MAPK) [11].

HER2 is a co-receptor for many different ligands and is often transactivated by EGF-like ligands, resulting in the formation of HER1–HER2 heterodimers [12, 13]. Neuregulins can induce the formation of HER2–HER3 and HER2–HER4 heterodimers [11, 14–16]. However, no known ligand can promote the HER2 homodimer (HER2–HER2), suggesting that no existing ligand binds directly to HER2. In support of this theory, analysis of representative pox virus growth factors, HER ligands of viral origin, revealed that none bind directly to HER2, but all are able to recruit HER2 into heterodimers [17]. This observation led us to propose that HER2 functions as a ligand-less receptor for many, if not all, HER ligands [18].

Inter-receptor interactions within the HER family are not random. Instead, there is a distinct hierarchy that prefers HER2 as an interaction partner [16]. An examination of the structure of one of the ligands helps to explain this preference. Neuregulin-1 (NDF) seems to be a bivalent molecule with two binding sites for HER receptors: a high affinity/narrow specificity site (N-terminal) and a low affinity with broad specificity site (C-terminal) [19]. The high affinity site binds first to its specific site (HER3 or HER4 receptor). Once the low affinity site is effectively ‘immobilized’ at the plasma membrane, its operational affinity to potential partners of the dimer is increased. Importantly, the receptor that preferentially binds to the immobilized low affinity arm of the ligand is HER2 (Figure 2). As a result of this heterodimerization, the HER2 receptor is able to participate in signal transduction in the absence of a specific ligand. This preferential binding with HER2 is also enhanced by the overexpression of HER2 in human cancer cells [8].

Heterodimers generate more potent signals than homodimers, and those containing HER2 have a particularly high ligand binding and signaling potency com-
Proliferation index

3.2 5.0 3.1 0 3.1 6.5 10.5

Figure 3 Relative potency of HER dimers. Note that HER4-containing combinations were omitted. The mitogenic index of each HER dimer was calculated on the basis of experiments described in Pinkas-Kramarski et al. [12]. (Reproduced by permission of Oxford University Press from Pinkas-Kramarski et al. [12].)

Figure 4. HER1–HER3 chimera is more potent than any other receptor combination. The presented erb-B1T3 artificial fusion protein was designed and tested in animal cells. Its activity exceeded that of the HER2–HER3 combination. (Reproduced by permission of Oxford University Press from Waterman et al. [24].)

In conclusion, signal transduction by the HER family may be considered in terms of information processing by networks. The network is a layered organization of units. Thus, the HER network consists of an input layer (ligands, growth factors), information processing layer (receptors, SH2-proteins, transcription factors), and an output layer (cell growth, differentiation or migration). Thus, receptor dimerization is important as it allows a signaling network with an enormous potential for diversification of biologic messages instead of four receptors with only four linear pathways. In effect, HER2 may be considered as a master regulator of the network because it promotes and controls signaling.

Negative regulation of HER receptors and its relevance to cancer immunotherapy

Of the 10 different HER homo- and heterodimer combinations, those containing HER2 are long-lived and transmit strong signals, and are thus associated with malignant growth (Figure 5) [5]. As mentioned earlier, overexpression of HER2 promotes the formation of more HER2 heterodimers. By contrast, the non-HER2 combinations signal relatively weakly. This signaling is normal, and these combinations are essential for normal cell growth but will not lead to tumor growth (Figure 5) [5].

In mice carrying human tumor cells overexpressing a rodent oncogenic mutant of HER2, the growth of tumors was inhibited by monoclonal antibodies directed at the HER2 receptor [25]. Tumor regression can be demonstrated also in a cell system that overexpresses the human HER2. When a number of different antibodies to HER2 were examined, the rate of tumor regression depended on type of antibody; some antibodies almost completely inhibited tumor growth, while others were only partial antagonists [26]. However, enhancement of growth also occurred upon treatment of cells with certain monoclonal antibodies. In these latter cases, labeling of tumor cells or the antibodies revealed that the tumor inhibitory antibodies were not processed by the tumor cells. By contrast, tumor-inhibitory antibodies were rapidly internalized and metabolized by...
cancer cells overexpressing HER2 [27]. These observations led us to suggest that tumor inhibition is due to the endocytic removal of HER2 from the cell surface [5]. Consistent with this model, combining two distinct antibodies to HER2 better inhibits tumors than each antibody alone, probably because the combination leads to accelerated degradation of HER2 inside tumor cells [28]. In conclusion, the anti-tumor effect of HER2-specific antibodies is due to the ability of certain antibodies to accelerate internalization and degradation of the oncprotein.

The attribution of immunotherapy to enhanced endocytosis focused the interest on processes leading to HER2 degradation. Generally, HER receptors are continuously recycled back to the cell surface after endocytosis through endosomes. Alternatively, the receptor may be degraded after passing from endosome to lysosome. The recycling/degradation pathway is controlled through an adaptor molecule, c-Cbl, which is a major tyrosine phosphorylation substrate of HER [29]. c-Cbl is the normal form of this sorting molecule and is needed for the HER1 receptor to move from endosome to lysosome and thus be degraded [30]. Unlike HER1, which is strongly coupled to c-Cbl, HER2 only weakly interacts with the adaptor, but the two neuregulin receptors, HER3 and HER4, do not recruit c-Cbl [31]. The biochemical basis of c-Cbl's action has been recently resolved. The ring finger domain of c-Cbl, a motif detected in two oncogenic forms of c-Cbl, serves as a binding site for a ubiquitin processing enzyme (E2).

Thereby, when c-Cbl is recruited to a specific tyrosine phosphorylated residue of HER1, it enhances polyubiquitination of the receptor (Figure 6). Polyubiquitin tracts are sufficient for targeting HER1 to proteasomal and lysosomal degradation [32]. It should be noted that HER3 is continuously recycled [33] because does not have an active kinase domain, nor does it carry a c-Cbl docking site. Our most recent results suggest that antitumor antibodies tag the HER2 receptor to degradation by recruiting c-Cbl and enhance ubiquitination of HER2.

Conclusions: Implications of HER2 biology for the treatment of breast cancer

In conclusion, the HER2 gene and HER2 receptor play a crucial role in the network of cell-signaling processes controlling normal growth and development. The HER family binds a range of ligands, and although HER2 does not bind a specific ligand, it is the preferred partner for receptor dimerization and this enables it to perform its role through growth factor binding at its partner receptor.

The role of HER2 in cell growth can be summarized in simple terms. When HER2 is normally expressed, ligands that bind to the HER receptors form only a few HER2 heterodimers, and the responses to these growth factors are relatively weak, resulting in normal growth of cells. However, when HER2 is overexpressed as in cancer cells, many ligands originating primarily in the
stoma or in the tumor cells themselves will recruit HER2 into heterodimers. The heterodimers stay longer at the cell surface, and their signaling is enhanced. This results in potent stroma-to-epithelium signaling, enhanced responsiveness to growth factors and, eventually, malignant growth.

In breast cancers, 92% of cases of HER2 overexpression result from HER2-gene amplification (generation of more than the normal two gene copies) [34]. This leads to increased transcription of the HER2 gene and concomitant increased synthesis of HER2 protein. In cancer cells, HER2 protein levels may be 100 times those in normal cells [35]. This is a consequence of gene amplification and/or transcriptional alterations. Amplification is also the most common cause of overexpression in ovarian and gastric tumors [4]. The mechanism of gene amplification has not been determined. However, the HER2 gene is not amplified in a few tumor types (lung, mesenchymal, bladder and esophageal) overexpressing HER2 and, in these cases, overexpression may result from transcriptional or post-transcriptional dysregulation [36].

As HER2 is frequently overexpressed in human tumors and often confers a more aggressive clinical course, HER2 is being investigated as a target for cancer therapy. Its localization at the cell surface makes it an easy target to access. A wide range of therapeutic strategies targeting breast tumors that overexpress HER2 have been investigated, including tyrosine kinase inhibitors, antisense approaches, designed to downregulate expression of the HER2 gene, and immunization to actively boost anti-HER2 responses. In addition, selective targeting can be achieved using monoclonal antibodies directed against the extracellular domain of the HER2 protein. As HER2 is expressed only in low levels in normal tissue [37], this permits a suitable therapeutic window to minimize damage to normal cells.

A number of murine monoclonal antibodies directed against the HER2 receptor have been developed. The monoclonal antibodies inhibited tumor growth in laboratory model systems. The mechanism of action of their anti-tumor effects is not yet completely understood although, as suggested earlier in this paper, the monoclonal antibodies may direct HER2 towards a degradation pathway. However, the murine antibodies caused the development of neutralizing human anti-mouse antibodies (HAMA) and did not progress beyond experimental stages. Since these experiments, the murine 4D5 monoclonal antibody has been humanized [38], resulting in the recombinant human anti-HER2 monoclonal antibody (rHuMAb-HER2, trastuzumab, Herceptin). This antibody is being introduced into clinical practice for patients with metastatic breast cancer. It is not associated with the development of HAMA and was effective in phase II and III clinical trials. In addition to being active as a single agent, trastuzumab potentiates the anti-tumor activity of paclitaxel and doxorubicin [39] and cisplatin [40].

Therefore, monoclonal antibodies are the potential greatest advance in the treatment of tumors overexpressing HER2. Elucidation of the role of HER2 in cell growth should allow the mechanism of action of monoclonal antibodies to be determined and should help optimize treatment of aggressive HER2-positive tumors.

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