Breast cancer is the most common cancer in women and the ErbB receptor family holds crucial role in its pathogenesis. Among them, epidermal growth factor receptor and HER-2 are the most studied members and their overexpression has been associated with aggressive clinical behaviour. These data were further strengthened by the clinical success of trastuzumab, a monoclonal antibody against HER-2 in breast cancer patients with HER-2 overexpression and/or amplification. However, trastuzumab failure in some patients may partly be attributed to co-expression of other ErbB receptors. Herein, we provide updated views regarding the role of HER-3 and HER-4 in breast cancer. Accumulated evidence implies that these receptors should be considered more than heterodimerisation partners. Their expression profile might be useful in predicting responsiveness to current treatment options, while new strategies targeting their ligands and downstream effectors are being developed.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Breast cancer; ErbB receptors; HER-3; HER-4

1. Introduction

The hallmark of breast cancer is the great heterogeneity rendering its treatment complex. Certain clinical characteristics have been used to predict which patients are likely to have favourable or unfavourable course. Molecular prognostic and predictive factors have been also extensively evaluated to assist tailored treatment of breast cancer more than for any other solid tumour (Karamouzis et al., 2002). However, the development of optimal consensus treatment guidelines for breast cancer requires both the comprehensive analysis of the results of randomized clinical trials and the interpretation of their clinical and biological relevance for individual patients (Goldhirsch et al., 2005). Molecular markers of prognosis and prediction in breast cancer are still considered less than ideal (Hayes, 2005). Recently, major research efforts have focused on identifying novel valuable markers, expression profiles and/or genetic “fingerprints” that could potentate the efficacy of current and future treatment regimens.

2. Pathogenesis

2.1. The ErbB receptor family in breast cancer

The transmembrane receptors that bear tyrosine kinase (TK) activity play a key role in breast cancer. The type I TK receptor family comprises four homologous members: ErbB-1 [epidermal growth factor (EGF) receptor (EGFR) or HER-1], ErbB-2 (HER-2/neu), ErbB-3 (HER-3) and ErbB-4 (HER-4). All family members have intrinsic TK activity, except for HER-3 (Fig. 1).
The phosphorylation of these receptors (evoked by ligand binding and homo- or heterodimerisation) ignites a cascade of signalling pathways that include various downstream adaptor and effector molecules that regulate pivotal cellular processes (Mosesson & Yarden, 2004) (Fig. 2). Despite their structural homology, ErbB receptors differ in their ligand specificities. Up to now, two main ligand classes have been identified, including the splice variants of heregulins (HRGs) and different EGF-related proteins. HRGs bind exclusively to HER-3 and/or HER-4, while a HER-2 ligand has not yet been identified (Harris, Chung, & Coffey, 2003) (Fig. 1).

HER-2 is known to be the preferred heterodimerisation partner for EGFR, HER-3 and HER-4. HER-2-containing heterodimers are more mitogenic than other ErbB combinations, probably due to their increased membrane stability and decreased degradation rates (Citri, Skaria, & Yarden, 2003). Notably, HER-2 through its interaction with heat shock protein 90 (Hsp90) has a unique regulation mode of its dimerisation and functional properties (Citri & Yarden, 2006) (Fig. 2). EGFR and HER-2 receptors are frequently overexpressed in breast cancer, and their expression has been associated with a more aggressive clinical behaviour and resistance to certain cytotoxic and endocrine therapies (Atalay, Cardoso, Awada, & Piccart, 2003). Most research efforts have focused on the evaluation of the expression and/or gene amplification of individual members, in relation to clinicopathological parameters. However, data concerning the role of HER-3 and HER-4 during breast carcinogenesis are limited and still controversial. Recent studies have highlighted the importance of the combined expression profile of all ErbB members in a large number of invasive and in situ breast carcinomas (Witton, Reeves, Going, Cooke, & Bartlett, 2003).

2.2. HER-3 and breast cancer

Among the ErbB family, HER-3 is inimitable because of its catalytically deficient kinase domain, its high propensity to self associate in the absence of ligand and the ability of the monomeric species of the extracellular domains of HER-3 to assume a locked conformation, using an intramolecular tether (Citri & Yarden, 2006). HER-3 gene is located on chromosome 12q13 and its encoded protein binds to HRG isoforms (Fig. 1). HER-3 signalling relies on the formation of signalling-competent heterodimers with other ErbB members. HER-3 has been found to be overexpressed in various organs including breast, lung, pancreas and stomach. Furthermore, its overexpression has been documented in 20–30% of invasive and in approximately one third of in situ breast carcinomas, and is associated with poor prognostic factors (Badra et al., 2006).

It has been demonstrated that the most mitogenic “couple” of ErbB receptors is HER-2/HER-3 (Citri et al., 2003). HER-2/HER-3 heterodimers have been shown to be constitutively active in breast cancer cells with HER-2 gene amplification. Simultaneous overexpression of HER-2 and HER-3 has been detected in 12–50% of inva-
Fig. 2. The ErbB receptors signalling modules. Ligand-bound ErbB homo-and/or heterodimers signal from the cell surface to the nucleus through a multipartite transduction system comprising a variety of adaptor and effector molecules. Ten possible dimers can be formed by ErbB receptors, but they do not all share the same efficacy. This richly layered network consists of mutually interacting protein cascades that ultimately convey their signal to TFs, which positively or negatively affect the transcription of target genes. Among these, signalling cascades are the stress-activated protein kinases pathway such as PLC–PKC and JAK–STAT, the RAS–RAF–MEK–MAPK pathway and the PI-3K–AKT pathway. The Cbl non-receptor TK is activated by ligand-stimulated EGFR/HER-3 dimers and regulates EGFR endocytosis. One exclusive HER-2 feature is its activity dependence on Hsp90 protein. ErbB receptors “transactions” differ based on the binding ligand, the dimer formed, the activated downstream signalling cascade and the cross-talk interactions with other intracellular pathways. This specificity is also reflected at the transcriptional control level and the eventual output signal, which may affect proliferation, differentiation, apoptosis, angiogenesis and metastasis. Hsp90, heat shock protein 90; JAK, janus kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/extracellular signal-regulated kinase (Erk) kinase; PAK, p21-activated kinase; PI-3K, phosphatidylinositol-3 kinase; PKB, protein kinase B; PKC, protein kinase C; PLC, phospholipase C; STAT, signal transducer and activator of transcription; TFs, transcription factors; TK, tyrosine kinase.

2.3. HER-4 and breast cancer

HER-4 gene is located on chromosome 2q33.3–34 and encodes a transmembrane glycoprotein that plays a crucial role in the development and differentiation of various tissues, especially cardiovascular, neural system and mammary glands as well as in several pathological processes, among them malignancy. HER-4 is considered both necessary and sufficient to trigger an anti-proliferative response in human breast cancer cells (Barnes et al., 2005). In contrast to HER-3, HER-4 can be activated by both HRGs and some of the EGF family of ligands (Fig. 1). Thus, there is considerable variability in receptor activation and potency as well as ligand diversity between HER-4 monomers and various HER-4-based heterodimers.

Specific features regarding HER-4 include different ligand–receptor interaction in various tissues and the identification of at least four splice variants of the protein. HER-4 has not been found to be frequently
overexpressed in breast carcinomas, compared with the other ErbB members. However, its expression has been associated with increased estrogen receptor (ER) positivity, low grade and cell proliferation rates. In cell line experiments, when HER-2 positive cancer cells were transfected to overexpress HER-4, a reduction in proliferation and an increase in apoptosis were observed, suggesting that HER-4 downsides HER-2 signalling activity (Barnes et al., 2005). Cell line studies have demonstrated that HRG-activated HER-4 homodimers stimulate only the apoptosis-controlling PI-3K–AKT pathway and not cell proliferation, probably reflecting a differentiation-related effect (Yarden & Sliwkowski, 2001). Moreover, novel mechanisms have been suggested for the HER-4-dependent apoptosis modulation (Naresh et al., 2006). Therefore, the paradoxical effect of HER-4 overexpression, which has been associated with good outcome, although not yet fully elucidated, might be explained by differences in the recruitment of signal transduction pathways and alternate apoptosis stimulation.

2.4. The combinatorial role of HRGs, HER-3 and HER-4 in breast cancer

HRG, also known as neuregulin or neu differentiation factor, acts as a preferential ligand for HER-3 and/or HER-4 receptors. Upon binding, HRG activates the EGFR/HER-2 receptors via dimerisation with HER-3 and/or HER-4. In mammary epithelial cells, HRG predominantly uses HER-2/HER-3 dimers to exert its biological effects (Tsai, Shamon-Taylor, Mehmi, Tang, & Lupu, 2003). A wide array of data reveals that HRG is sufficient for generation of breast carcinomas and favours their metastatic spread (Marshall, Blackburn, Clark, Humphreys, & Gullick, 2006). Blockade of HRG in vitro inhibits tumourigenesis of breast cancer cells (Tsai et al., 2003), while its expression (in ~30% of breast cancers) has been correlated with poor prognostic features (Menendez, Mehmi, & Lupu, 2006). However, the underlying mechanisms of HRG cytoplasmic signalling leading to gene expression are not fully elucidated. It has been postulated that HRG activates both the RAS–MAPK and PI-3K–AKT signalling cascades (Muraoka-Cook et al., 2006). Subsequent work by many groups has shown that HRG is expressed as multiple isoforms that share variable potency and receptor specificity. HRG3 is a soluble form of HRG, whilst other isoforms are at least initially membrane bound (Fig. 1).

Recent reports suggest that HER-3- and HER-4-overexpressing breast tumours may represent different subtypes of breast cancer. HER-2 deregulation occurs early during breast carcinogenesis, while EGFR overexpression is considered a late event. In invasive breast carcinomas, a positive association of HER-3 and an inverse association of HER-4 with the other ErbB receptors have been described (Abd El-Rehim et al., 2004; Witton et al., 2003). Nevertheless, in earlier stages of breast carcinogenesis, an opposite correlation has been documented (Badra et al., 2006). Thus, a possible scenario might be that HER-3 and HER-4 co-expression pattern along with the expression of EGFR and/or HER-2 represent a crucial intracellular molecular switch regarding ErbB dimer formation, and the prevalent signal transduction module in earlier stages of breast tumourigenesis (Fig. 3). It should be noted, however, that diverse molecular pathways (e.g., ERs and other signalling molecules) are implicated in breast carcinogenesis, depending on the cell type and differentiation state.

3. Therapy

In recent years, significant treatment advances parallel the increased detection of breast carcinomas in earlier and more curable stages. In conjunction to the refinement of conventional treatment modalities, the identification of HER-2 as an important regulator of breast cancer cell proliferation led to the development of strategies aiming at reducing HER-2 receptor levels or activity, with the more successful being trastuzumab. However, certain aspects related to trastuzumab await clarification. Its limited efficacy in some patients inspired the notion that other factors, besides HER-2 status, might be required for breast cancer cell proliferation. For example, the co-expression profile of ErbB receptors might be responsible for the enhanced transforming potential and worsened prognosis. Therefore, identification of inhibitor(s) targeting alternate or multiple members of the ErbB family, either at the extracellular level or at their downstream signalling cascades might provide improved therapeutic efficacy.

Research is now under way to develop novel molecules that block EGFR, EGFR and HER-2 and/or the entire ErbB family. The first clinical studies of EGFR TK inhibitors (TKIs) in breast cancer patients with metastatic disease refractory to multiple previous treatments, produced poor clinical results (Roy & Perez, 2006). A more effective strategy would be to apply dual-specificity inhibitors for both EGFR and HER-2 (e.g., lapatinib, EKB-569) (Citri & Yarden, 2006). Initial clinical trials with the use of lapatinib in women with HER-2 positive, advanced breast cancer with progressive disease despite trastuzumab treatment, yielded promising
Breast Carcinogenesis

Fig. 3. Proposed model for the implication of HER-3 and HER-4 in breast cancer evolution and possible intervention strategies. Breast carcinogenesis represents a multistep process, via a series of intermediate hyperplastic (with or without atypia) and preinvasive stages until the formation of invasive carcinomas. It seems plausible that in the early stages of this process HER-4 overexpression dominates over HER-3 expression, favoring the formation of HER-4-containing dimers and the preferential activation of this receptor by its cognate ligands. This could lead to enhanced apoptosis and net control of aberrant proliferation caused by HER-2. However, the accumulation of genetic and epigenetic defects causes an inversion of the HER-3 to HER-4 expression ratio, which combined with EGFR (HER-1) overexpression, results in inhibition of apoptosis and triggering of cellular proliferation. This might also be supported by the inherent propensity of HRGs to activate HER-3-containing dimers and the increased utilization of EGF-related peptides by EGFR. In this molecular and phenotypic course, multiple potential targeting points exist (indicated by stars) for the prevention and/or treatment of breast carcinogenesis. AR, amphiregulin; BC, betacellulin; EGF, epidermal growth factor; EGFR, EGF receptor; EPR, epiregulin; HB-EGF, heparin-binding EGF-like ligand; HRG, heregulin; TGF-α, transforming growth factor-α; TK, tyrosine kinase.

results (Nelson & Dodler, 2006). There are also a number of TKIs that are potent in vitro inhibitors of all ErbB receptors and are currently appraised (e.g., CI-1033). “Pan-HER” inhibition may be a potential treatment approach for a broad variety of breast cancers expressing different levels and subclasses of ErbB receptors (Xu et al., 2005). However, a rational caveat of “pan-HER” blockade might be that HER-4 downregulation could alleviate the positive effects of its signalling pathway. Other evolving treatment strategies target recently elucidated intracellular processes that affect ErbB receptors functionality (e.g., modulators of endocytosis and degradation, PI-3K/AKT modifiers) (Fig. 3).

The role of ligands in breast cancer development has been less investigated. HRG causes autocrine constitutive activation of signalling cascades, such as Ras–MAPK and PI-3K–AKT, which have been linked to breast cancer cell proliferation. HRG-dependent activation of HER-2, HER-3 and HER-4 has also been associated with increased breast cancer cell sensitivity to cytotoxics. However, it remains to be addressed whether HRG-induced activation of HER-2, via activation of HER-2-containing dimers, can cause the same biologic effects as HER-2 overexpression itself. Importantly, it has been shown that the population of breast tumours that overexpress HRG is distinct from that exhibiting HER-2 overexpression. Furthermore, a recent study suggested that HRG expression characterizes a breast cancer subpopulation, for which trastuzumab and chemotherapy are effective without the prerequisite of HER-2 overexpression (Menendez et al., 2006). The cell type-specific effects of HRG might be related to the expression and activation levels of ErbB receptors. Because, HRG causes HER-2 tyrosine phosphorylation indirectly through its binding to HER-3 and HER-4, the ligand could deliver its signal singly via HER-4 or through complexes containing combinations of HER-2, HER-3 and HER-4.
Taken together, these findings strongly support the concept that the HRG, HER-3 and HER-4 molecular interplay might represent an attractive area for the application of novel targeting strategies for breast cancer prevention and/or treatment, depending on the active forms of ErbB receptors, the preferred dimer formation and the activation status of downstream transduction cascades in various stages of breast carcinogenesis (Fig. 3).

References


