Chapter 1

Introduction and background biology

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Introduction

The successful targeting of growth factor receptors is one of the most fruitful areas of new drug discovery and development in recent years. A key moment in this chapter of modern pharmacology is the outstanding results obtained from targeting the receptor tyrosine kinase (RTK) coded by the *ERBB2* gene (also known as human epidermal growth factor receptor 2 [HER2]) with the humanized monoclonal antibody trastuzumab in women with HER2 overexpressing/amplified breast cancer. The basis for developing one of the emblematic therapeutic strategies of modern oncology stands on the original observation that amplification of HER2 was linked to a poorer outcome than recorded in non-amplified cases of breast cancer. It took almost two decades from that seminal article to the establishment of trastuzumab as standard of therapy for HER2-positive breast cancer, and the accompanying demonstration that there are a sizeable number of breast carcinomas that are 'addicted' to HER2 signaling and therefore are unable to survive a block or modulation of the signaling pathway(s) downstream of the receptor. In the following chapter, we will cover some key aspects of the biology and pathology of HER2 in breast cancer.

Epidemiology

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in women worldwide, accounting for 25% (1.67 million) of the total new cancer cases and 15% (522,000) of the total cancer deaths in 2012 [1]. More than half of all cases and 60% of the deaths are estimated to occur

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in economically developing countries [1]. The mortality for breast cancer has been decreasing over the past 25 years, largely as a result of early detection through mammography and improved treatment [2–5]. In fact, in Europe and USA, most breast cancers are diagnosed when the tumor is still confined to the breast and can be treated with curative intent. However, breast cancer remains a major cause of death in women aged between 35 and 59 years of age.

About 15–25% of breast cancers overexpress HER2 [6,7], which belongs to a family of transmembrane RTKs that mediate cell growth, differentiation, and survival [8,9]. HER2 overexpression is associated with aggressive tumor behavior [6] and until the advent of HER2-targeted therapies, patients affected by HER2-positive early breast cancer faced a poorer prognosis than patients with HER2-negative disease, including reduced relapse-free and overall survival, with a peak of recurrence at 2–3 years from diagnosis [10,11]. In addition, approximately 50% of ductal carcinomas in situ (DCIS) display HER2 amplification. The concept that a portion of DCIS eventually evolve into HER2 overexpressing infiltrating carcinomas is still the focus of discussion [12,13].

HER2 overexpression has a well defined association with prognosis. In addition, since the advent of trastuzumab and other HER2-targeting drugs, preclinical and clinical studies have shown that in women with advanced breast cancer the clinical benefit of HER2-targeting therapies are limited to those breast cancers that display the highest levels of overexpression [14], which is almost fully concordant with gene amplification. This clinical evidence has led to the routine testing of breast cancer for HER2 as a predictive factor to guide the therapeutic decision process [15].

HER2 primary structure

HER2 is a 185 KD glycoprotein encoded by a gene localized on the long arm of chromosome 17 (17q12-21) and is normally expressed in the epithelia of various organs such as lung, bladder, pancreas, breast and prostate [16,17]. It belongs to the ErbB family of transmembrane RTKs, which are a subclass of cell-surface growth-factor receptors with an intrinsic, ligand-controlled tyrosine kinase activity. RTKs have a crucial role in the signaling pathways that govern key cellular processes, such as proliferation, migration, metabolism, differentiation, and survival, and signaling that regulates intercellular communication during development. RTK activity in normal cells is tightly controlled. Mutations or

structural alterations, however, cause abnormal activation of RTKs, which become potent oncoproteins involved in the development and progression of many human cancers.

The ErbB family of receptors, to which HER2 belongs, includes four members: EGFR (epidermal growth factor receptor, also known as ErbB1), HER2, HER3, and HER4 (human EGFR-related-2, -3, and -4, named for their high level of homology to human EGFR; also named ErbB2, ErbB3, and ErbB4). These receptors are characterized by a similar molecular structure, composed of an extracellular ligand-binding domain (ECD), a hydrophobic transmembrane region and an intracellular tyrosine kinase portion. The latter domain comprises an extended C-terminal tail that includes the adenosine triphosphate (ATP)-linking position for receptor autophosphorylation and phosphorylation of respective substrates [18,19].

The ligands of the ErbB RTKs are the epidermal growth factor (EGF) for EGFR and primarily neuregulins (NRG1–4) for HER3 and HER4. HER2 has no ligand binding site and it is still unknown if a ligand for HER2 exists; HER3 does not possess kinase activity and the receptor can initiate signal transduction only when dimerized with another HER2 family member (Figure 1.1) [20].

Signal transduction through receptor tyrosine kinases

The ErbB proteins stand on the surface of the plasma membrane in an inactivated state and are activated by ligand binding. The ligand-bound RTKs undergo a conformational change of the extracellular domain that induces the dimerization of the receptors in homodimers and heterodimers, if the dimerization involves the same receptors or two different receptors, respectively. Dimerization of the receptor(s) causes autophosphorylation of the tyrosine residues of the catalytic kinase domains, which results in the activation of intracellular tyrosine-kinase cascades responsible for the downstream signal transduction [21].

Unlike other ErbB family members, HER2 lacks a ligand binding site, is constitutively active, and can undergo ligand-independent dimerization. Importantly, HER2 is the preferred partner for the other ErbB proteins, and heterodimers containing HER2 are more potent in signal transduction than homodimers of HER2 or homodimers of other ErbB proteins. In particular, the combination of HER2 and HER3 is very potent in the activation of survival and proliferation networks

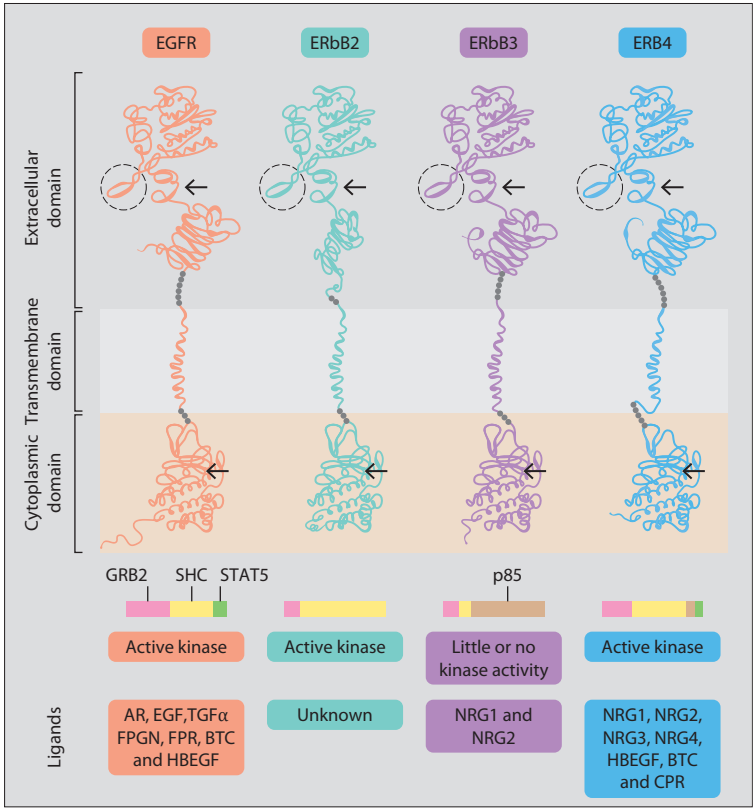

Figure 1.1 The four members of the epidermal growth factor receptor family. These are represented by their corresponding and highly homologous crystal structures. They have three major domains: the ligand-binding domain, the transmembrane domain and the kinase domain. The ligand-binding clefts are marked by upper arrows and the dimerization loops by dashed circles. ErbB2 has no ligand-binding cleft. Bottom arrows mark the ATP-binding sites. AR, amphiregulin; BTC, β-cellulin; EGF, epidermal growth factor; EPGN, epigen; EPR, epiregulin; HBEGF, heparin-binding EGF-like growth factor; NRG, neuregulin; STAT5, signal transducer and activator of transcription 5; TGFα, transforming growth factor-α. Reproduced with permission from Yarden and Pines [20] ©Nature Publishing Group.

because the cytoplasmic tail of HER3 contains binding sites for phosphatidylinositide 3-kinases (PI3K), which strongly activates the PI3K-Akt-mTOR (mammalian target of rapamycin) pathway, whereas HER2 powerfully signals through the mitogen-activated protein kinase (MAPK) pathway. The subsequent deregulation of PI3K and MAPK signalling strongly enhances cell proliferation and evasion of apoptosis [22–25]. Therefore, the overexpression of HER2 that occurs in HER2-positive breast

cancer can directly result in excess dimerization of ErbB proteins and subsequent increase of cellular signaling, resulting in a poor clinical outcome in breast cancer and resistance to various cancer therapies.

Receptor tyrosine kinases: site of therapeutic intervention

The observation that deregulation of the RTK-signaling network is crucial for tumor growth and survival constitutes the rationale for the development of targeted anticancer therapies. Neutralizing antibodies, which block the bioactivity of RTK ligands, RTK-targeted antibodies, which target overexpressed receptors, and small-molecule inhibitors of RTK kinase activity have been developed to interfere with RTK signal transduction.

An overview of the HER2-signaling network and the therapeutic strategies directed against HER2 are summarized in Figure 1.2 and Figure 1.3.

Signaling through HER-receptor family dimers leads to the activation of downstream cascades

The signaling and metabolic networks involving HER2 are characterized by great plasticity that confers robustness to the system through amplification and redundancy of signals, but also fragility through feedback loops that elicit resistance to anti-HER2 agents [26]. Two main mechanisms of resistance have been identified so far: HER2 can evade the targeted drug, or the driving role of HER2 in cancer 'addiction' is taken over by another pathway [27,28]. HER2 may elude trastuzumab (but not lapatinib or other HER 2-targeted tyrosine kinase inhibitors) by alternative splicing or proteolytic cleavage, which generates an intracellular constitutively active fragment called p95 [29], or changes in the structure of the ECD, which prevent the antibody-receptor interaction and the consequent immune response (antibody-dependent cell-mediated cytotoxicity [ADCC]) [30,31]. In addition, compensatory pathways such as upregulation of ErbB3 or insulin-like growth factor 1 receptor (IGF1R) or activation of the PI3K-Akt pathway through loss of *PTEN* or PI3K mutation have been described in trastuzumab-resistant model systems [32–36]. Similarly, resistance of breast cancer cells to lapatinib may involve overexpression of other RTKs or de-repression of the estrogen receptor (ER) pathway [37,38]. A more recent analysis showed that mutations in exon 21 of the *HER2* gene are also involved

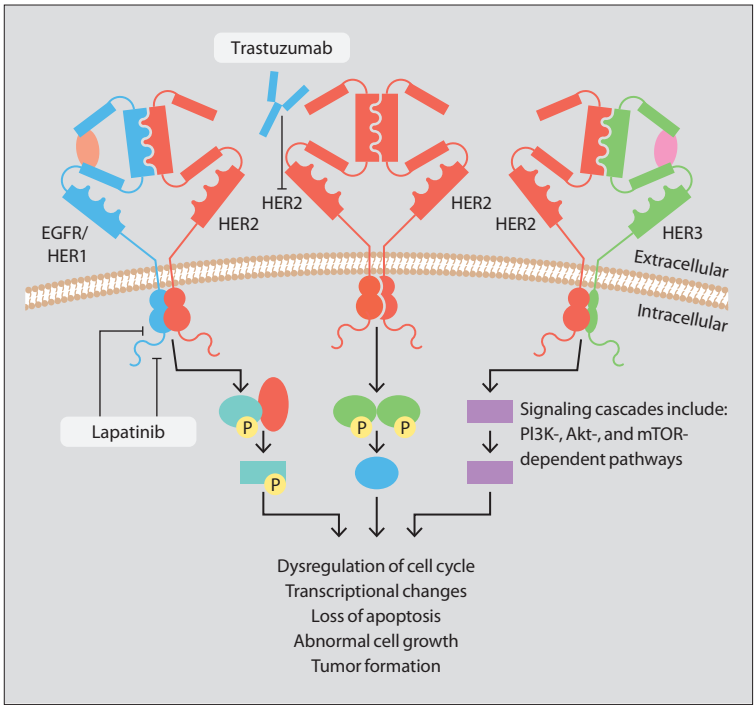

Figure 1.2 Signaling through HER-receptors family dimers leads to the activation of downstream cascades. Downstream signaling pathways include PI3K-, Akt-, mTOR- and MAPK-pathways, which control cell cycle, cell growth and survival, apoptosis, metabolism and angiogenesis. Signaling through HER2 homodimers is inhibited by the monoclonal antibody trastuzumab. Lapatinib is a small molecule that inhibits HER1 and HER2 tyrosine kinase activities. Akt, protein kinase B; EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; mTOR, mammalian target of rapamycin. Adapted from Ahn and Vogel [24].

in resistance to trastuzumab, without directly affecting trastuzumab binding to the receptor because exon 21 codes for the intracellular tyrosine kinase domain and mutations in this region do not change the structure of HER2 ECD [39].

Other molecules currently being investigated in clinical trials of HER2-resistant breast cancer include Heat Shock Protein 90 (HSP90) inhibitors and telomerase inhibitors. Indeed, HSP90 acts as a chaperone protein which promotes the stabilization of many other proteins, including HER2, and prevents their rapid degradation. Telomerase expression is crucial for cellular proliferation and telomerase overexpression has been linked to tumorigenesis, whereas the inhibition of telomerase results in apoptosis or cell senescence. In trastuzumab-resistant cell lines, the inhibition of HSP90 or the inhibition of

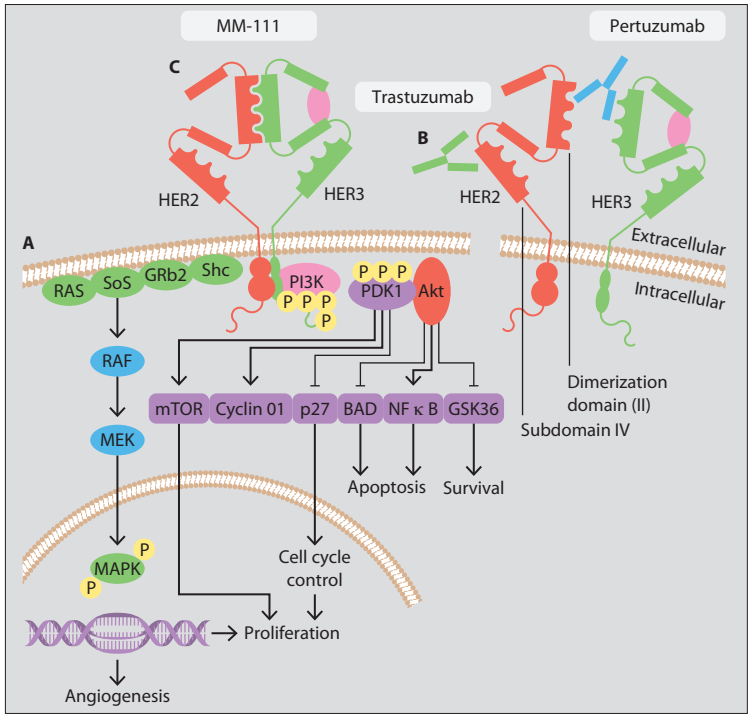

Figure 1.3 The HER2:HER3 heterodimer. (A) The HER2:HER3 heterodimer is a potent trigger of downstream signaling cascades, especially the PI3K/Akt cascade and the MAPK cascade. **(B)** HER2:HER3 signaling can be inhibited by the monoclonal antibody pertuzumab, preventing HER2:HER3 dimer formation by blocking the HER2 dimerization domain (subdomain II), which is distinct from the site of trastuzumab binding (subdomain IV). (**C**) A bispecific antibody for both HER2 and HER3 (MM-111) is being evaluated in combination with trastuzumab. Akt, protein kinase B; HER, human epidermal growth factor receptor; GRb2, growth factor receptor-bound protein 2; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog. Adapted from Ahn and Vogel [24].

telomerase was able to restore trastuzumab sensitivity [40,41]. Furthermore, several lines of evidence recently showed that the crosstalk between the ER and HER2 pathways plays a role in resistance to HER2-directed agents [42,43]. In fact, signaling from EGFR can downregulate ER and, conversely, the inhibition of HER2 with either trastuzumab or lapatinib, results in upregulation of ER and increased transcription of ER-regulated genes, which act as an 'escape' mechanism that contributes to resistance to HER2-directed agents [44]. This observation suggests that the combined inhibition of ER and HER2 may be critical to prevent the development of resistance to HER2-targeted therapies and

that the identification of mechanisms of resistance to trastuzumab has important implications for the rational selection of subsequent targeted therapies against other pathways and molecules implicated in HER2 resistance (Figure 1.4) [45].

The great plasticity of the HER2-regulated network and the many functions played by the different intracellular and extracellular domains of the HER2 receptors have led to the concept that dual targeting of HER2 may lead to enhanced therapeutic results in HER2-overexpressing tumors. Preclinical evidence in the KPL4 model of trastuzumab-resistant breast cancer have clearly shown that the combined use of trastuzumab with the monoclonal antibody pertuzumab, which blocks receptor dimerization, was more active than either monoclonal antibody alone. In addition, evidence was provided that tumor regrowth after initial response to trastuzumab could be reversed upon introduction of pertuzumab as second antibody [46]. The clinical evidence collected over the years is in line with the evidence obtained from animal models. Introduction of pertuzumab while continuing trastuzumab led to a high rate of objective responses and long-lasting stable disease in women with HER2-positive breast

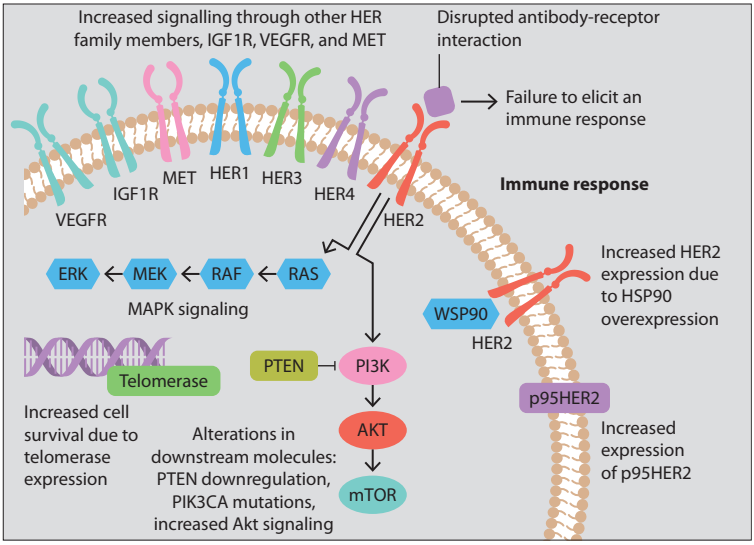

Figure 1.4 Proposed mechanisms of HER2 resistance. Akt, protein kinase B; HER, human epidermal growth factor receptor; HSP90, heat shock protein 90; IGF1R, insulin-like growth factor receptor 1; MAPK, mitogen-activated protein kinase; MET, mesenchymal epithelial transition factor; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; VEGFR, vascular endothelial growth factor receptor. Reproduced with permission from Mohd Sharial et al [45] ©Oxford University Press.

cancer progressing on trastuzumab either alone or in combination with chemotherapy [47]. This led to the initiation of the neoadjuvant study NEOSPHERE, which showed a significantly higher rate of pathologic eradication of operable breast cancer with the combination of pertuzumab and trastuzumab with docetaxel than with the conventional combination of trastuzumab and the taxane [48]. Conclusive evidence of the superiority of this approach to dual blockade of HER2 has been provided by the Phase III trial CLEOPATRA, which showed superior response, progression-free survival and overall survival with trastuzumab, pertuzumab and docetaxel compared with trastuzumab and docetaxel in HER2-positive metastatic breast cancer [49,50].

In a different approach to dual targeting, the tyrosine kinase inhibitor lapatinib was combined with trastuzumab. This approach leads to a more complete inhibition of receptor signaling while maintaining the immune mechanisms of activity afforded by the immunoglobulin (IgG) nature of trastuzumab. In addition, lapatinib should also block the signaling of truncated forms of HER2, for which trastuzumab is devoid of any activity. The preclinical evidence in favor of combining trastuzumab with a HER2 tyrosine kinase inhibitor found clear-cut correspondence with the clinical findings of NeoALLTO, a neoadjuvant study in which lapatinib and trastuzumab with paclitaxel were compared with trastuzumab and paclitaxel or lapatinib and paclitaxel [51]. The results showed a significant benefit for the dual HER2 targeting approach.

The success of treating HER2-positive breast cancer with HER2-targeted drugs with different mechanisms of action confirms that superior results can be expected by inhibiting the HER2-associated signaling pathway at different points. This is consistent with results from preclinical studies showing a potential benefit from concomitant use of trastuzumab and inhibitors of the PI3K [52], or inhibitors of mammalian target of rapamycin (mTOR) [53,54].

The immune system and response to HER2-targeted treatment

A growing body of preclinical and clinical evidence shows that the innate and adaptive immune system contributes substantially to the therapeutic effects of trastuzumab in vivo [55,56]. A correlation has been noted between a higher level of immune infiltration and a lower risk of relapse in patients not receiving

adjuvant treatment, irrespective of molecular subtype. The most consistent association between good prognosis and immune infiltration has been recorded in triple-negative and HER2-positive tumors [57–59]. Study findings in patients with breast cancer treated with neoadjuvant chemotherapy show that augmented expression of immune-associated genes and extensive lymphocyte infiltration in the tumor before treatment are associated with increased likelihood of a pathological complete response in HER2-positive tumors [60]. Furthermore, findings indicate that immune-related markers can provide useful predictive information and that increased clinical activity might follow activation of the immune system. Development of immunomodulatory drugs with remarkable activity in many solid tumors defines a scenario in which the combination of immune modulation with trastuzumab, or other HER2-directed drugs, will result in augmented response and clinical outcome [61–64].

Conclusions

The thorough characterization of HER2 biology and the involvement of this growth factor receptor in the pathogenesis and maintenance of about 25% of breast carcinomas has contributed to the development of one of the most successful therapeutic interventions in the era of targeted oncology drugs. In addition, it has contributed to the elucidation of mechanisms relevant to other therapeutic approaches and other neoplastic diseases. Importantly, it has clearly shown the power of the concomitant targeting of the HER2 pathway in HER2 'addicted' carcinomas, illustrating the benefit of a multipronged treatment approach with targeted agents in oncology. For all the above, the clarification of HER2 biology and pathology and the still ongoing development of multiple targeted approaches against HER2-driven tumors stands as a reference in the field of modern oncology.

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