J. Cell. Mol. Med. Vol 19, No 12, 2015 pp. 2691-2701

Analysis of different HER-2 mutations in breast cancer progression and drug resistance

Zijia Sun [a,#], Yaqin Shi [a,#], Yan Shen [a], Lulu Cao [a], Wenwen Zhang [a, *], Xiaoxiang Guan [a, b, *]

[a] *Department of Medical Oncology, Jinling Hospital, Medical School of Nanjing University, Nanjing, China*
[b] *Department of Medical Oncology, Jinling Hospital, School of Medicine, Southern Medical University, Guangzhou, China*

Received: April 29, 2015; Accepted: July 3, 2015

Abstract

Studies over the last two decades have identified that amplified human epidermal growth factor receptor (HER-2; c-erbB-2, neu) and its overexpression have been frequently implicated in the carcinogenesis and prognosis in a variety of solid tumours, especially breast cancer. Lots of painstaking efforts were invested on the HER-2 targeted agents, and significantly improved outcome and prolonged the survival of patients. However, some patients classified as 'HER-2-positive' would be still resistant to the anti-HER-2 therapy. Various mechanisms of drug resistance have been illustrated and the alteration of HER-2 was considered as a crucial mechanism. However, systematic researches in regard to the HER-2 mutations and variants are still inadequate. Notably, the alterations of HER-2 play an important role in drug resistance, but also have a potential association with the cancer risk. In this review, we summarize the possible mutations and focus on HER-2 variants' role in breast cancer tumourigenesis. Additionally, the alteration of HER-2, as a potential mechanism of resistance to trastuzumab, is discussed here. We hope that HER-2 related activating mutations could potentially offer more therapeutic opportunities to a broader range of patients than previously classified as HER-2 overexpressed.

Keywords: breast cancer • HER-2 • HER-2 mutation • variants • cancer risk • resistance

Introduction

HER-2 is a member of the human epidermal growth factor receptor (HER) family, additionally comprised of epidermal growth factor receptor (EGFR), HER-3, and HER-4. These receptors regulate normal cell proliferation, survival, and differentiation *via* different signal transduction pathways [1]. The gene encoding HER-2 is located in chromosome 17, and codes for a 185-kPa protein that functions as a transmembrane growth factor receptor [2]. The intracellular domain of HER-2 contains approximately 500 residues and composed of three parts: a cytoplasmic juxtamembrane linker, a tyrosine kinase (TK) domain and a carboxyl-terminal tail [3, 4]. The TK domain is more complicated than other parts of HER-2 receptor, which contains several important loops: the C-loop (residues 844–845), the αC-helix (residues 761–775), the N-loop (residues 727–732) and the activation loop (A-loop residues 863–884), to form the enzyme active site [3].

Though HER-2 point or insertion mutations were first described in 2004, researches efforts about them are not exhaustive compared with his family EGFR to date [5]. According to the existing data, the probability of HER-2 mutations is 1.67% in breast cancer, 1–4% in lung cancer and 2.9% in colorectal [6–12]. Other human tumour types have also been reported to harbour HER-2 mutations, including

[#]Zijia Sun and Yaqin Shi should be regarded as joint first authors for their equal contributions.
*Correspondence to: Prof. Xiaoxiang GUAN
E-mail: xguan@nju.edu.cn

Dr. Wenwen ZHANG
E-mail: wwzhang1022@hotmail.com

doi: 10.1111/jcmm.12662

head and neck cancers, bladder cancers, gastric cancers, ovarian cancers, hepatic cancers [6, 9–21]. Mutational activation of HER-2 can result from three types of somatic molecular alterations: small insertions and missense mutations in the kinase domain, missense mutations in the extracellular domain, or large deletions of the extracellular domain which yield a truncated form of HER-2 [22, 23]. More mutations are mainly located in the three exons (19–21) of the TK domain [24], and are encoded by the DNA sequences in the exons 18–23 [25]. HER-2 kinase domain mutations have been described in lung carcinoma and breast cancer albeit at a lower frequency [26–29]. HER-2 kinase domain mutations can be categorized as: missense point mutations, small in-frame insertions or duplications which almost occurring in exon 20 and in frame deletions. Among these mutations, the in-frame insertions or duplications in exon 20 are the most frequently encountered types of mutations [22, 30–32]. In addition, we also take the HER-2 splice variants into account, including p95HER-2 and Δ16HER-2.

The clinical success of gefitinib, an inhibitor of EGFR, in a subset of lung cancers with mutations in the TK domain of EGFR, holds a promise for the future of targeted therapy [33, 34], and also leads to the investigation of analogous mutations of HER-2. With the application of HER-2 fluorescent *in situ* hybridization and HER-2 immunohistochemistry which are standard clinical tests for HER-2 gene amplification [35, 36], HER-2 gene amplification or protein overexpression has been extensively studied in breast cancer [37–40], much less is known about genetic variants and mutations that might have an impact on the risk or therapy of breast cancer. It may be more challenged to successfully target HER-2 mutations than EGFR mutation. More efforts are needed to translate this idea to clinic.

The HER-2 mutations and variants

The HER-2 mutations

These HER-2 mutations are the common type found in the patients lacking HER-2 overexpression and most of them were found in the TK domain (Fig. 1).

Mutations in TK domain

Human epidermal growth factor receptor-2 gene amplification or protein overexpression has been identified as a mechanism of HER-2 activation in breast cancer [1]. However, HER-2 activating mutations, another novel modus to activate HER-2, have been reported [41, 42]. Bose and his colleagues identified 16 HER-2 somatic mutations though cancer genome sequencing in HER-2 gene amplification-negative breast cancer patients. Seven of these HER-2 kinase domain mutations are activating and oncogenic, including G309A, D769H, D769Y, V777L, P780ins, V842I and R896C [23]. Activating HER-2 kinase domain mutations could also been found at low frequency in several other carcinomas, such as bladder cancer and lung cancer [23, 31, 43].

Human epidermal growth factor receptor-2 gene with some kinase domain mutations shows the characteristics of constitutively activate kinase activity and increased oncogenicity compared to the wild-type HER-2 [44]. The positive effect of activating mutations on tumour growth has been demonstrated *in vitro* [23]. The enhanced kinase activity promotes the formation of the dimmers. Meanwhile, the activating mutations particularly induce the phosphorylation of cellular signalling proteins [23].

The most prevalent activating mutations of HER-2 involve the insertions within exon 20 and these mutations have stronger catalytic activity [44]. These insertions are more potent in transphosphorylating EGFR compared with the wild-type HER-2. Conclusively, the mutant HER-2 gene is more transforming and more capable to inhibit the effect of apoptosis [44].

For the small insertion activating mutations, the basic mechanism is that these mutations lead to a conformational change of the autoinhibition, consequently keeping their inactive condition. The oncogenic insertions frequently alter the Adenosine triphosphate (ATP)-binding cleft, which forms a conformational structure with many important structures surround the cleft, including phosphate-binding and activation loops [45].These insertions induce a conformational change of the autoinhibitory αC-β4 loop, thus, narrowing the ATP-binding cleft, increasing both the ATP binding affinity and turnover number, and promoting the enhanced kinase activity that participates in the subsequent phosphorylation events [46]. In addition, the HER-2 insertions can potently transphosphorylate EGFR, even in the presence of EGFR tyrosine kinase inhibitors (TKI) [44]. It induced transphosphorylation of kinase-dead EGFR, and exhibited higher ligand-independent tyrosine phosphorylation. Moreover, the insertions were more potent than wide-type HER-2 in associating with signal transducers that mediate proliferative and prosurvival responses [44].The activating missense mutations in kinase domain have also been reported. Most of the mutations were detected in the αC-helix which is considered to play a critical role in the activation of HER-2 gene [23]. The structures of αC-helix may be altered by the single missense mutations, and these altered structures might promote tumourigenesis and the phosphorylation of signalling proteins including (phospholipase γ C1, phospholipase Cγ (PLCγ) and mitogen-activated protein kinase(MAP Kinase)) [25]. Some other mutations, such as the HER- exon 19 in-frame deletions 755–759, homologous to EGFR exon 19 in-frame deletions, could potently increase phosphorylation of EGFR or HER-3, and interact differently with its dimerization partners compared with other HER-2 mutants [25]. In addition to these activating mutations, some mutations are resistant to the targeted therapy, such as mutations located at codon 755 or 798. The underlying mechanisms of resistance will be discussed in the next sections.

HER-2 mutations in other domain

Apart from the mutations in TK domain, recurring HER-2 extracellular domain mutations in breast and lung cancer were also identified (*e.g.* S310F/Y, G309A/E, S335C) [23, 47–50]. Parts of these mutations that cluster in exon 8 are oncogenic and activated by two distinct mechanisms, characterized by elevated C-terminal tail phosphorylation, such as S310F/Y, or covalent dimerization mediated by intermolecular disulfide bond formation, such as G309E and S335C [47]. These extracellular domain mutations are also sensitive to small-molecule inhibitors of HER-2, more similar to the kinase domain mutations

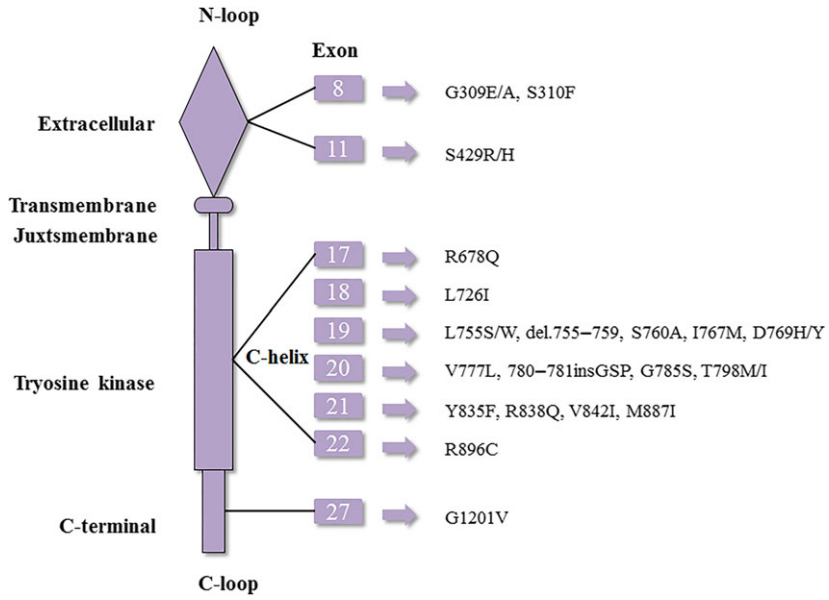

Fig. 1 The HER-2 mutations. These HER-2 mutations are the common type found in the patients lacking HER-2 expression and most of them were found in the tyrosine kinase domain. HER: human epidermal growth factor receptor.

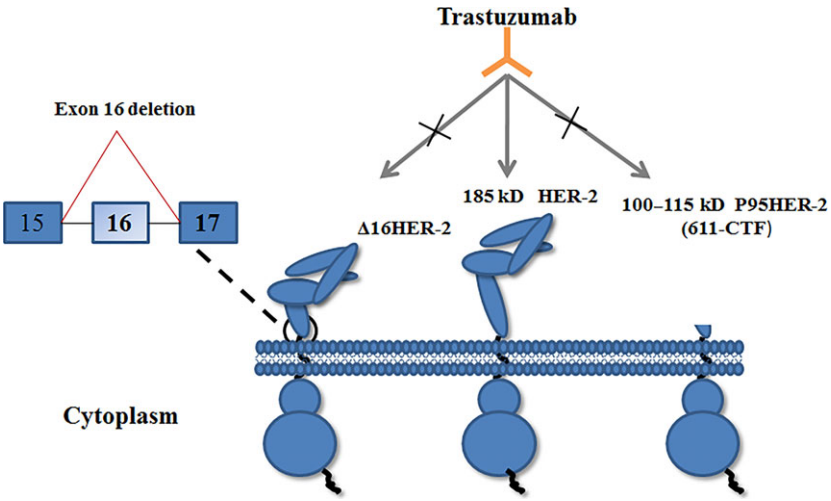

Fig. 2 The structure of the Δ16HER-2 and p95HER-2 variants. The variants, different from the point mutations, can be defined as incomplete HER-2 or fragments of HER-2, including Δ16HER-2 and p90HER-2. HER: human epidermal growth factor receptor.

[23]. Trastuzumab was shown to be effective against the cells expressing G309 and S310 mutations, giving hope to the patients harbouring these mutations [47].

Recently, novel transmembrane domain mutations were also reported in familial lung adenocarcinoma, including kinds of germline mutations (G660D, V659E and I655V). The V659E was first detected in a case report of Li-Fraumeni syndrome, and was found to have a oncogenic role [51]. G660D and V659E mutations in the transmembrane domain also correlate with the hereditary, sporadic lung adenocarcinomas [52]. The G660D and V659E mutations, more stable than wild-type genes, both can act as driver mutations in lung cancer, and have the capacity to activate Akt. Simultaneously, p38 was also activated to promote cell proliferation in lung adenocarcinoma [52]. Additionally, I655V in the transmembrane domain was reported to increase the breast cancer risk.

The HER-2 variants

These variants, different from the point mutations, can be defined as incomplete HER-2 or fragments of HER-2. They may even appear opposite functions due to their various constructions (Fig. 1).

Δ16HER-2

Except for the small insertions or point mutations, some other resistant HER-2 alterations have also been identified in HER-2-positive breast cancer, such as Δ16HER-2 (a HER-2 splice variant lacking exon 16) and p95HER-2 (carboxy-terminal HER-2 fragments, mostly known as 611-CTF; Fig. 2) [53, 54]. Both of these alterations could explain the clinical failure of trastuzumab [55–60]. The Δ16HER-2, a type of oncogenic variant caused by the in-frame deletion of exon 16 in the extracellular domain of HER-2, is reported to comprise 4–9%

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

of total HER-2 transcripts [56, 61]. The Δ16HER-2 variant, when expressed at high levels, harbours enhanced transforming activity compared with wild-type HER-2 [54]. The primary activating mechanism is that this conformation with removed relevant cysteine residues promotes intermolecular disulfide bonding and the generation of homodimers, thus transforming cells [62]. Another study found that 44% of Δ16HER-2-expressing breast cancer showed the activated Src kinase, heralding the potential clinical implications of direct coupling of Δ16HER-2 to Src kinase. The activated Src kinase is also associated with the metastatic tumour, suggesting that more aggressive therapeutic interventions are needed [54]. Δ16HER-2 has also been implicated in resistance of HER-2 positive breast cancers to anti-HER-2 therapies. Thus, measurement of this variant may help predict the response to treatment of anti-HER-2 therapy. However, to our knowledge, no studies were conducted on the issue till now [63, 64].

p95HER-2

p95HER-2 is a form of truncated HER-2, which does not have the complete extracellular domain (Fig. 2). This CTF are yielded through at least two different mechanisms: proteolytic shedding of the extracellular domain of the full-length HER-2 receptor or translation of HER-2 mRNA from alternate internal initiation codons (positions 611 and 678, respectively) [65, 66]. Strikingly, *in vitro* studies of 611-CTF (100–115 kD) revealed more rapid activation of multiple signalling pathways to promote tumour progression when compared with the full length receptor and 648-CTF [67]. p95HER-2 is hyperactive and has been demonstrated to play a role in cancer progression, increased metastasis, poor prognosis and disease-free survival when compared with patients that express the wild full-length HER-2 [65, 68]. Approximately 30% of HER-2-positive tumours express this HER-2 fragment [66]. The specific characteristic of p95HER-2 is still unclear, but the overexpression of p95HER-2 can promote the growth of tumour *via* forming homodimers by intermolecular disulfide bonds in subdomain IV, similar to dimers formed in extracellular domain mutations [69, 70].

Because p95 is thought to be sensitive to HER-2 active TKI, measurement of quantitative p95 levels may have potential role of treatment decisions in future. Till now, at least two antibodies have been generated against the 611-CTF form of p95 [63, 64]. One of the antibodies, D9, has been generated to build a quantitative p95 assay to identify a group of HER-2 positive patients expressing p95HER-2 that have a worse outcome while on trastuzumab.

Some other variants of HER-2

Other variants of HER-2 with contrasting roles in tumour have also been detected, such as Herstatin (results from intron 8 retention) and p100 (results from intron 15 retention) [71]. These variants can interfere with the oncogenic activity of wild-type HER-2, to inhibit tumour cell growth [56]. Further exploration of p100 found a decrease in downstream signal induction. Besides, the protective Herstatin have also been reported to inhibit the activity of HER-2 by interfering with the phosphorylation of dimmers (HER-2/HER-3 and HER-2/EGFR) [56]. Herein, considering of the less association with breast cancer risk or drug resistance, we will not focus on these two subsets of variants more.

The HER-2 mutations associated with breast cancer risk

A breadth of literature describes the link between genetic variations and breast cancer risk. Single nucleotide polymorphisms (SNPs) are the commonest sources of human genetic variations that contribute to a susceptibility of tumour progression [72]. A polymorphism of the HER-2 gene that results in the substitution of isoleucine-to-valine atcodon 655 of the transmembrane domain (Ile655Val, rs1136201) has been extensively investigated as a risk factor for breast cancer [73]. Since the initial case–control study on 700 Han chinese women from Xie *et al.* reported a significantly increased risk for carriers of this allele [odds ratio (OR) = 1.4] [74], many epidemiological studies have been conducted to reveal an association between the HER-2 655V polymorphism and an increased risk of breast cancer [31]. However, the results are inconsistent. Several meta-analyses have been performed to investigate the association between the polymorphism and breast cancer. Due to the differential including studies and methodological issues, different results arise from these meta-analyses. Lu *et al.* found a significant association among Africans and Asians, but not in Europeans [75]. However, Ma *et al.* did not demonstrate any significant associations between HER-2 codon 655 polymorphism and breast cancer susceptibility, either at the overall or the ethnicity analyses [76]. Moreover, a novel updated meta-analysis suggests that this polymorphism is marginally associated with breast cancer in worldwide populations with additive and dominant models, but not a recessive model [77]. Thus, no confirmed associations could be identified between the polymorphism and an increased breast cancer risk among different ethnicities.

A stronger association was revealed in women both under the age of 45 years and with a family history, and the valine allele might not have any effect among women older than 60 [78]. Additionally, it also raised a possibility that the risk associated with carrying the HER-2 valine allele might predominantly affect pre- or peri-menopausal breast cancer [78]. Consistent with the above investigations, another study also suggested that that V/V or V/I genotype have a twofold increased risk compared with I/I genotype among women who were both younger than 45 years of age and reported a positive family history of breast cancer (OR = 2.3, 95% CI = 1–5.3) [79]. However, some conflicting results emerged, revealing that HER-2 I655V polymorphism may be a biomarker for breast cancer susceptibility among older women [80]. Furthermore, a rare HER-2 variant Ile654Val is also associated with an increased familial breast cancer risk, which revealed an oncogenic role for carriers of the heterozygous Val654 allele (OR = 2.56, 95% CI = 1.08–6.08, $P = 0.028$) [81], meanwhile, it is linked with the more frequent Val655 to form two consecutive valines instead of two isoleucine residues [81].

Human epidermal growth factor receptor-2 is considered an orphan receptor since it is the only receptor of HER family in the absence of an identified ligand (Fig. 3) [82, 83]. However, HER-2-

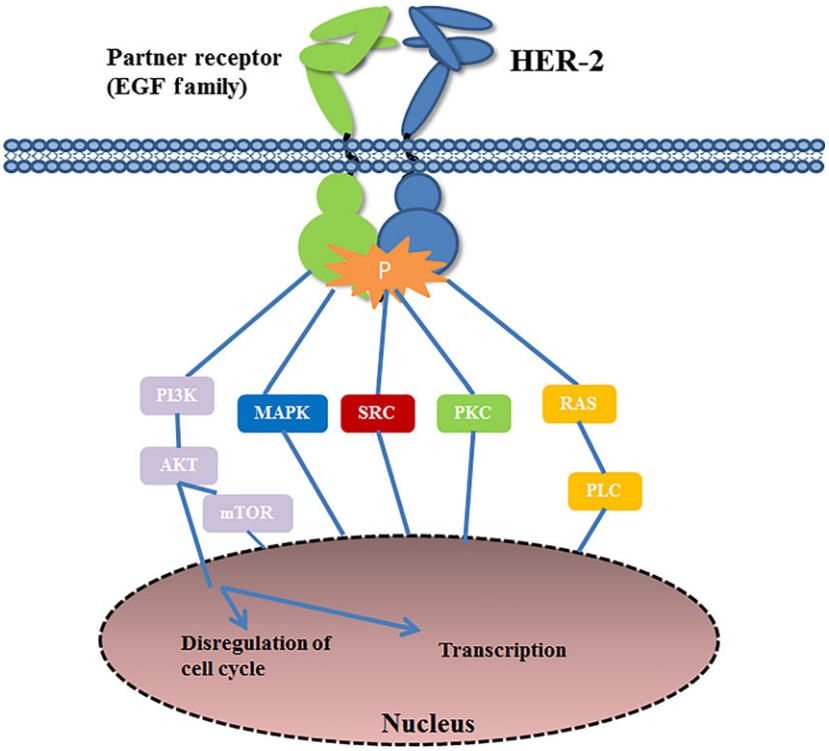

Fig. 3 Working model for the human epidermal growth factor receptor (HER)-2 oncogenic activities in breast cancer development and progression.

containing heterodimers could function as the most active signalling complex of the HER family [84, 85]. A strong pro-tumourigenic signalling cascade is initiated by the overexpression of HER-2, and leads to the generation of dimmers [86, 87]. A subsequent activation of HER-2 cytoplasmic kinase activity is needed for the downstream PI3K/AKT signalling pathways to promote cell proliferation and the effect of apoptosis (Fig. 3) [85, 87, 88]. The basic activating mechanism of HER-2 variants is to enhance the kinase activity and cell transformation by increasing the formation of active HER-2 heterodimmers [72]. The implication of HER-2 polymorphism in tumour progression may preferentially occur through the modification of function rather than the amplification of protein [72]. Furthermore, the independent genetic variant in growth factors signalling appear to have the stronger influence on breast cancer risk *via* combining with other variant gene, including variant fibroblast growth factor 1 (FGF1), FGF2 and neuregulin 2 (NRG2), interacted with SNPs in platelet-derived growth factor B (PDGFB), EGFR, HER-2 and FGFR2 [89].

The HER-2 mutations associated with breast cancer resistance

In 1987, HER-2 amplification and overexpression were first reported. These molecular alterations could be found in approximately 20–30% of breast cancer [90]. Since then, HER-2 was considered as a significant targeted point due to its distinctive role in tumour cell proliferation and metastasis, facilitating the development of HER-2 targeted agents, which have shown a tremendous success [91]. However, the efficacy of anti-HER-2 therapeutics such as trastuzumab or small molecule HER-2 TKI (lapatinib) is limited by the occurrence of therapeutic resistance [92, 93]. Major mechanisms of primary or acquired resistance against these targeted agents include [94]: (*i*) Alteration in binding sites or TK receptor domain. (*ii*) Up-regulation of alternative ErbB ligands and dimerization of receptors to counteract for receptor inhibition. (*iii*) Dimerization/interaction with other receptors. (*iv*) Downstream controllers-deficient tumours. (*v*) Activation of downstream signalling and survival pathways. Here we mainly discuss the alteration of HER-2 gene (Table 1).

The point or small insertion resistance mutations

To our knowledge, HER-2 point or small insertions were found predominantly in patients lacking HER-2 amplification to date [24]. It suggests that the mutant gene may not be associated with real amplification [95]. In fact, insufficient evidence about the mutations in HER-2 'positive' can be searched. Among these limited researches, rare patients harbouring HER-2 amplification were tested for the presence of mutations. Hence, it is an arduous work to obtain any conclusions about relative characteristics. Herein, we conclude some possible reasons that could explain this low frequency event, including: one possible reason is that these mutations may be acquired only after the utilization of anti-HER-2 agents, suggesting that the anti-HER-2 agents might be a trigger to the generation of resistance alterations. Another possible explanation is that these mutations only

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

Table 1 The HER-2 mutations and variations associated with breast cancer resistance

Mutations	Primary tumour	Number of patients screened	TNM stage	Drug resistance	Reference
L726I	Cell study	NA	NA	Gefitinib	[104]
L726F	NA	76	NA	Lapatinib	[42]
L726F	Cell study	NA	NA	Lapatinib	[101]
L755S	Invasive ductal	94	IIIA	Lapatinib	[7]
L755S	Invasive ductal	94	IIA	Lapatinib	[7]
L755S	Lobular	193	NA	Lapatinib	[28]
L755S	TNBC	104	NA	Lapatinib	[29]
L755S	Lobular	1499	IIA	Lapatinib	[23]
L755S	Ductal	1499	I	Lapatinib	[23]
L755P	Cell study	NA	NA	Lapatinib	[103]
P780L	Cell study	NA	NA	Lapatinib	[101]
S783P	Cell study	NA	NA	Lapatinib	[101]
L785F	Cell study	NA	NA	Lapatinib	[101]
T798M	Cell study	NA	NA	Lapatinib/Trastuzumab	[97]
T798M	Cell study	NA	NA	Lapatinib	[103]
T798I	Cell study	NA	NA	Lapatinib	[101]
T798I	Cell study	NA	NA	Lapatinib	[102]
p95HER-2	NA	483	NA	Trastuzumab	[68]
Δ16HER-2	NA	NA	NA	Trastuzumab	[62]

NA: not available; TNBC: triple negative breast cancer; HER: human epidermal growth factor receptor.

occupy small parts of the amplified HER-2, thereby, may not be detected by the DNA sequencing methods due to below the limits of sensitivity [95]. Further investigations are needed to clarify the specific mechanisms.

These unusual intrinsic mutations in HER-2 overexpressed patients occur with an inconspicuous probability. It is reported that a 52-year-old man diagnosed with stage IV non-small cell lung cancer (NSCLC), was detected to overexpress HER-2 and harbour an L869R mutation. Later he achieved a partial response to lapatinib, but showed no response to trastuzumab alone. The mutation in this patient is analogue to the previously reported L861 mutation in EGFR [7, 96]. But whether the resistance is caused by the mutation or not is still unknown. Another study revealed that the HER-2 gene-amplified breast cancer cells, which harbour the T798M mutant alleles, acquired a resistance to both lapatinib and trastuzumab alone. However, after the treatment of a simultaneous blockade of HER-2 and EGFR, an effective response was shown, hinting a possible connection between increased EGFR ligand production and drug resistance [97]. Additionally, it should be noted that T798 is a gatekeeper residue, analogous to the gatekeeper EGFR T790M [98], ABLT315I [99] and cKITT670I [100] mutations [97], which are all related to the clinical drug resistance. All above results indicate that the amino acids L755 and T798 in HER-2 are critical residues, enable to determine lapatinib sensitivity. Strong lapatinib resistance caused by L755S, L755P and T798M, T798I have been reported [101, 102]. The frequency of T798 mutations is higher than other mutations [23, 103]. Despite the resistance mutation, activating mutation (D769H) have also been detected in HER-2 positive patients. But whether some associations exist between activating mutations and HER-2-positive patients is unclear yet [23].

Apart from the above conditions, the mutations could also occur after the treatment of anti-agents. The wild-type HER-2 could acquire the L755S and T862A mutation after the exposure of lapatinib, suggesting that kinase domain mutations may cause a secondary resistance in patients with wild type HER-2 [103]. Bose and his colleagues identified that L755S mutation was the most common subtype of mutant HER-2 in breast cancer. In his study, six of total 27 patients with mutant HER-2 were detected with the L755S mutation [23].

Similar condition occurred after the treatment of other agents, such as gefitinib or iressa, a selective epidermal growth factor receptor TKI, primarily for NSCLC, also respond to breast cancer with positive HER-2. A subset of breast cancer cell lines overexpressing the activated HER-2 was treated with 5 Mmol/l gefitinibt. Then a novel point mutation-L726I in the ATP-binding pocket was found, enabling these cells insensitive to gefitinib [104].

Most HER-2 mutations associated with lapatinib resistance locate in the ATP-binding and hinge region. Herein, we mainly discuss the mechanism of L755 and T798 due to their vital roles. Mutations at L755 can conclusively stabilize the active conformation of the HER-2 kinase. They may not directly affect inhibitor binding, but form a conformation where the αC-helix is fixed in to inhibit lapatinib binding and influence the structure of the active state [103]. As the HER-2 'gatekeeper', T798 is located in exon 20 within hinge region, which is the most prominent site of resistance mutations. Mutations at T798 may lead to the obstacle of TKI by directly interfering with the steric structure [101]. One mechanism is to increase the affinity of HER-2-T798M towards ATP, similar to the T790M mutation in EGFR. Another mechanism is that lapatinib binds the inactive conformation preferentially, then incapable to bind the active conformation in T798M [103]. Additionally, another studies revealed that cells harbouring T798M mutation showed increased expressions of the EGFR ligands EGF, transforming growth factor-α, amphiregulin and proheparin-binding epidermal growth factor (HB-EGF), leading to the resistance of TKI [97]. Besides, the ability of HER-2YVMA's mutation to amplify their transforming potential and modify tumour microenvironment through induction of growth factors was recently demonstrated [105].

The resistance variants of HER-2

Human epidermal growth factor receptor-2 variants were identified in patients with HER-2 amplification, and conferred resistance to targeted therapy. These variants mainly include Δ16HER-2 and p95HER-2.

The patients harbouring this Δ16HER-2 are also refractory to the treatment of trastuzumab. The mechanism of this clinical failure still needs exploration. The potential oncogenic properties were mediated through a direct interaction between Δ16HER-2 and Src kinase. Treatment with single-agent TKI dasatinib overcame the resistance to trastuzumab, and suppressed tumourigenicity. In addition, the capacity of stabilizing HER-2 homodimers and the phosphorylated state of phosphatase and tensin homolog (PTEN) may also contribute to the resistance [54]. Surprisingly, trastuzumab was even identified to promote the growth and invasion of tumour cell [54]. Interestingly, it also demonstrated that 89% of patients with the expressed Δ16HER-2 were locally disseminated node-positive breast cancer, indicating that more attention should be put on this subtype [56].

p95HER-2 is hyperactive, and was described as a truncated form of HER-2 lacking the antibody's binding region. It has been demonstrated to be associated with cancer progression, metastasis, poor prognosis and disease-free survival when compared with patients expressing the wild full-length HER-2 [65, 68]. Additionally, patients with breast cancer harbouring the expression of p95HER-2 exhibit less response to trastuzumab compared to patients without p95HER-2. The lack of outer-cell attachment domain containing the binding site for trastuzumab results in the failure of drug binding. Besides, actively signalling protein generated by ectopic expression of p95HER-2, also promotes trastuzumab resistance that has been demonstrated in preclinical and clinical studies [95].

The therapy methods to these HER-2 mutations

Recently, more researches about the HER-2 mutations have been investigated. Most of the mutations were described in patients without HER-2 overexpression or amplification. These have brought us a profound change to the traditional targeted therapy for patients with mutant HER-2. Studies about HER-2 mutations, both *in vitro* and *in vivo*, have proved that the applicability of anti-HER-2 agents, such as inhibitor combinations, lapatinib plus trastuzumab, or afatinib plus rapamycin, are the most effective therapy in HER-2-mutant cancers [106, 107]. Patients with HER-2-mutant NSCLC were also reported to have achieved disease control with anti-HER-2 therapy [108, 109]. Similar outcome of HER-2-targeted therapy has also been achieved in breast cancer patients with HER-2-mutantions [103, 110]. Notably, in Bose's research, all HER-2 mutations including mutation L755S which is resistant to lapatinib, exhibited sensitivity to the irreversible HER-2 inhibitor, neratinib [23]. All these findings validate that patients with HER-2-mutant could benefit from existing HER-2 targeted therapy, particularly irreversible inhibitors, such as neratinib. More preclinical and clinical trials should be designed to investigate the application of HER-2 targeted therapy in HER-2 mutation positive patients. To date, a prospective, multicenter clinical trial is being launched to screen patients with metastatic breast cancer for HER-2 mutation and investigate the clinical outcome of HER-2 targeted therapy (NCT01670877).

Previous researches have revealed that tumours contain p95HER-2 are resistant to trastuzumab, but still sensitive to the TKI, such as lapatinib [53]. In addition, some studies also reported that these TKIs could also provide an effective solution for the patients expressing mutant Δ16HER-2, suggesting that the TKI may be an alternative therapy for HER-2 mutations [56]. Furthermore, another study indicated that p95HER-2 could be a possible biomarker to evaluate the efficacy of therapeutic regimens including lapatinib and chemotherapy, and overcome the clinical failure of trastuzumab monotherapy. The effect of lapatinib, as single agents or in combination with other drugs, may be equal in patients regardless of the p95HER-2 expression [70]. Interestingly, a study revealed that tumours expressing these p95HER-2 fragments, also respond to trastuzumab plus chemotherapy, advocating that p95HER-2 is also a predictive biomarker for the patients treated with trastuzumab and chemotherapy [53]. However, no inhibitors were directly targeted against p95HER-2. To improve the outcome of patients harbouring these two variants, new researches are still on the way.

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

Conclusion

During the past decades, the application of trastuzumab, which targeted against HER-2, has significantly improved the outcome and prognosis of HER-2-overexpressing breast cancer. However, despite the clinical success, the resistance of HER-2 targeted agents occurred. Primary activating mutations and acquired secondary mutations were detected to play a critical role in breast cancer progression and drug resistance, revealing a sophisticated challenge for the effective treatment of HER-2 targeted therapy. Lots of preclinical data suggest that the combination of multipoints HER-2 targeted therapy may break the drug resistance. This will require an 'individualized diagnosis and treatment' based on the detailed molecular analysis of tumours both before and after progression on primary HER-2 targeted therapy. Future works are still needed to evaluate the role of altered HER-2 as future prognostic and predictive factors, as well as potential therapeutic targets, and providing 'an individualized strategy' for patients with HER-2 mutant.

Acknowledgments

This research was supported by grants from the National Natural Science Foundation of China (no. 81272252 and 81470357), a Foundation for Clinical Medicine Science and Technology Special Project of the Jiangsu Province, China (no. BL2014071 to X. G).

Conflicts of interest

The authors do not have any financial interests to publish this article.

References

1. **Iqbal N, Iqbal N.** Human epidermal growth factor receptor 2 (HER2) in cancers: overexpression and therapeutic implications. *Mol Biol Int*. 2014; 2014: 852748.
2. **Brandt-Rauf PW, Pincus MR, Carney WP.** The c-erbB-2 protein in oncogenesis: molecular structure to molecular epidemiology. *Crit Rev Oncog*. 1994; 5: 313–29.
3. **Telesco SE, Radhakrishnan R.** Atomistic insights into regulatory mechanisms of the HER2 tyrosine kinase domain: a molecular dynamics study. *Biophys J*. 2009; 96: 2321–34.
4. **Lemmon MA, Schlessinger J.** Cell signaling by receptor tyrosine kinases. *Cell*. 2010; 141: 1117–34.
5. **Stephens P, Hunter C, Bignell G, *et al.*** Intragenic ERBB2 kinase mutations in tumours. *Nature*. 2004; 431: 525–6.
6. **Yan M, Parker BA, Schwab R, *et al.*** HER2 aberrations in cancer: implications for therapy. *Cancer Treat Rev*. 2014; 40: 770–80.
7. **Lee JW, Soung YH, Seo SH, *et al.*** Somatic mutations of ERBB2 kinase domain in gastric, colorectal, and breast carcinomas. *Clin Cancer Res*. 2006; 12: 57–61.
8. **Sasaki H, Shimizu S, Endo K, *et al.*** EGFR and erbB2 mutation status in Japanese lung cancer patients. *Int J Cancer*. 2006; 118: 180–4.
9. **Ding L, Getz G, Wheeler DA, *et al.*** Somatic mutations affect key pathways in lung adenocarcinoma. *Nature*. 2008; 455: 1069–75.
10. **Minami Y, Shimamura T, Shah K, *et al.*** The major lung cancer-derived mutants of ERBB2 are oncogenic and are associated with sensitivity to the irreversible EGFR/ERBB2 inhibitor HKI-272. *Oncogene*. 2007; 26: 5023–7.
11. **Gilmer TM, Cable L, Alligood K, *et al.*** Impact of common epidermal growth factor receptor and HER2 variants on receptor activity and inhibition by lapatinib. *Cancer Res*. 2008; 68: 571–9.
12. **Suzuki M, Shiraishi K, Yoshida A, *et al.*** HER2 gene mutations in non-small cell lung carcinomas: concurrence with her2 gene amplification and her2 protein expression and phosphorylation. *Lung Cancer*. 2015; 87: 14–22.
13. **De Greve J, Teugels E, Geers C, *et al.*** Clinical activity of afatinib (BIBW 2992) in patients with lung adenocarcinoma with mutations in the kinase domain of HER2/neu. *Lung Cancer*. 2012; 76: 123–7.
14. **Del Campo J, Hitt R, Sebastian P, *et al.*** Effects of lapatinib monotherapy: results of a randomised phase II study in therapy-naive patients with locally advanced squamous cell carcinoma of the head and neck. *Br J Cancer*. 2011; 105: 618–27.
15. **Bang Y-J, Van Cutsem E, Feyereislova A, *et al.*** Trastuzumab in combination with chemotherapy *versus* chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet*. 2010; 376: 687–97.
16. **Lin W-L, Kuo W-H, Chen F-L, *et al.*** Identification of the coexisting HER2 gene amplification and novel mutations in the HER2 protein-overexpressed mucinous epithelial ovarian cancer. *Ann Surg Oncol*. 2011; 18: 2388–94.
17. **Lassus H, Sihto H, Leminen A, *et al.*** Gene amplification, mutation, and protein expression of EGFR and mutations of ERBB2 in serous ovarian carcinoma. *J Mol Med*. 2006; 84: 671–81.
18. **Bekaii-Saab T, Williams N, Plass C, *et al.*** A novel mutation in the tyrosine kinase domain of ERBB2 in hepatocellular carcinoma. *BMC Cancer*. 2006; 6: 278.
19. **Tschui J, Vassella E, Bandi N, *et al.*** Morphological and molecular characteristics of HER2 amplified urothelial bladder cancer. *Virchows Arch*. 2015; 466: 1–8.
20. **Kubo T, Kuroda Y, Shimizu H, *et al.*** Resequencing and copy number analysis of the human tyrosine kinase gene family in poorly differentiated gastric cancer. *Carcinogenesis*. 2009; 30: bgp206.
21. **Cohen EE, Lingen MW, Martin LE, *et al.*** Response of some head and neck cancers to epidermal growth factor receptor tyrosine kinase inhibitors may be linked to mutation of ERBB2 rather than EGFR. *Clin Cancer Res*. 2005; 11: 8105–8.
22. **Arcila ME, Chaft JE, Nafa K, *et al.*** Prevalence, clinicopathologic associations, and molecular spectrum of ERBB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. *Clin Cancer Res*. 2012; 18: 4910–8.
23. **Bose R, Kavuri SM, Searleman AC, *et al.*** Activating HER2 mutations in HER2 gene

amplification negative breast cancer. *Cancer Discov*. 2013; 3: 224–37.
24. **Matthew J. Mutational analysis of breast cancer: Guiding personalized treatments.** *Breast*. 2013; 22: S19–21.
25. **Lee JW, Soung YH, Kim SY, *et al.*** ERBB2 kinase domain mutation in the lung squamous cell carcinoma. *Cancer Lett*. 2006; 237: 89–94.
26. **Tomizawa K, Suda K, Onozato R, *et al.*** Prognostic and predictive implications of HER2/ERBB2/neu gene mutations in lung cancers. *Lung Cancer*. 2011; 74: 139–44.
27. **Kelly RJ, Carter CA, Giaccone G.** HER2 mutations in non–small-cell lung cancer can be continually targeted. *J Clin Oncol*. 2012; 30: 3318–9.
28. **Shah SP, Morin RD, Khattra J, *et al.*** Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution. *Nature*. 2009; 461: 809–13.
29. **Shah SP, Roth A, Goya R, *et al.*** The clonal and mutational evolution spectrum of primary triple-negative breast cancers. *Nature*. 2012; 486: 395–9.
30. **Buttitta F, Barassi F, Fresu G, *et al.*** Mutational analysis of the HER2 gene in lung tumors from Caucasian patients: mutations are mainly present in adenocarcinomas with bronchioloalveolar features. *Int J Cancer*. 2006; 119: 2586–91.
31. **Shigematsu H, Takahashi T, Nomura M, *et al.*** Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. *Cancer Res*. 2005; 65: 1642–6.
32. **Shimamura T, Ji H, Minami Y, *et al.*** Non-small-cell lung cancer and Ba/F3 transformed cells harboring the ERBB2 G776insV_G/C mutation are sensitive to the dual-specific epidermal growth factor receptor and ERBB2 inhibitor HKI-272. *Cancer Res*. 2006; 66: 6487–91.
33. **Lynch TJ, Bell DW, Sordella R, *et al.*** Activating mutations in the epidermal growth factor receptor underlying responsiveness of non–small-cell lung cancer to gefitinib. *N Engl J Med*. 2004; 350: 2129–39.
34. **Mitsudomi T, Morita S, Yatabe Y, *et al.*** Gefitinib *versus* cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol*. 2010; 11: 121–8.
35. **Pauletti G, Godolphin W, Press MF, *et al.*** Detection and quantitation of HER-2/neu gene amplification in human breast cancer archival material using fluorescence *in situ* hybridization. *Oncogene*. 1996; 13: 63–72.
36. **Kamoshida S.** Challenges of immunohistochemistry for individualized cancer chemotherapy. *Rinsho Byori*. 2014; 62: 710–8.
37. **Perez EA, Romond EH, Suman VJ, *et al.*** Trastuzumab plus adjuvant chemotherapy for human epidermal growth factor receptor 2–positive breast cancer: planned joint analysis of overall survival from NSABP B-31 and NCCTG N9831. *J Clin Oncol*. 2014; 32: 3744–52.
38. **Slamon DJ, Leyland-Jones B, Shak S, *et al.*** Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med*. 2001; 344: 783–92.
39. **Romond EH, Perez EA, Bryant J, *et al.*** Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med*. 2005; 353: 1673–84.
40. **Baselga J, Cortés J, Im S-A, *et al.*** Biomarker analyses in CLEOPATRA: a phase III, placebo-controlled study of pertuzumab in human epidermal growth factor receptor 2–positive, first-line metastatic breast cancer. *J Clin Oncol*. 2014; 32: 3753–61.
41. **Weigelt B, Reis-Filho JS.** Activating mutations in HER2: neu opportunities and neu challenges. *Cancer Discov*. 2013; 3: 145–7.
42. **Boulbes DR, Arold ST, Chauhan GB, *et al.*** HER family kinase domain mutations promote tumor progression and can predict response to treatment in human breast cancer. *Mol Oncol*. 2014; 9: 586–600.
43. **Ross JS, Wang K, Gay LM, *et al.*** A high frequency of activating extracellular domain ERBB2 (HER2) mutation in micropapillary urothelial carcinoma. *Clin Cancer Res*. 2014; 20: 68–75.
44. **Wang SZE, Narasanna A, Perez-Torres M, *et al.*** HER2 kinase domain mutation results in constitutive phosphorylation and activation of HER2 and EGFR and resistance to EGFR tyrosine kinase inhibitors. *Cancer Cell*. 2006; 10: 25–38.
45. **Fang Y, Jiang Y, Wang X, *et al.*** Somatic mutations of the HER2 in metastatic breast cancer. *Tumour Biol*. 2014; 35: 11851–4.
46. **Herter-Sprie GS, Greulich H, Wong K-K.** Activating mutations in ERBB2 and their impact on diagnostics and treatment. *Front Oncol*. 2013; 3: 86.
47. **Greulich H, Kaplan B, Mertins P, *et al.*** Functional analysis of receptor tyrosine kinase mutations in lung cancer identifies oncogenic extracellular domain mutations of ERBB2. *Proc Natl Acad Sci USA*. 2012; 109: 14476–81.
48. **Kan ZY, Jaiswal BS, Stinson J, *et al.*** Diverse somatic mutation patterns and pathway alterations in human cancers. *Nature*. 2010; 466: 869–U103.
49. **Vornicova O, Hershkovitz D, Yablonski-Peretz T, *et al.*** Treatment of metastatic extramammary Paget's disease associated with adnexal adenocarcinoma, with anti-HER2 drugs based on genomic alteration ERBB2 S310F. *Oncologist*. 2014; 19: 1006–7.
50. **Cancer Genome Atlas Research Network**. Integrated genomic analyses of ovarian carcinoma. *Nature*. 2011; 474: 609–15.
51. **Serra V, Vivancos A, Puente XS, *et al.*** Clinical response to a lapatinib-based therapy for a Li-Fraumeni syndrome patient with a novel HER2V659E mutation. *Cancer Discov*. 2013; 3: 1238–44.
52. **Yamamoto H, Higasa K, Sakaguchi M, *et al.*** Novel germline mutation in the transmembrane domain of HER2 in familial lung adenocarcinomas. *J Natl Cancer Inst*. 2014; 106: djt338.
53. **Parra-Palau JL, Morancho B, Peg V, *et al.*** Effect of p95HER2/611CTF on the response to trastuzumab and chemotherapy. *J Natl Cancer Inst*. 2014; 106: dju291.
54. **Mitra D, Brumlik MJ, Okamgba SU, *et al.*** An oncogenic isoform of HER2 associated with locally disseminated breast cancer and trastuzumab resistance. *Mol Cancer Ther*. 2009; 8: 2152–62.
55. **Barić M, Kulić A, Sirotković-Skerlev M, *et al.*** Circulating her-2/neu extracellular domain in breast cancer patients-correlation with prognosis and clinicopathological parameters including steroid receptor, her-2/neu receptor coexpression. *Pathol Oncol Res*. 2014; 21: 1–7.
56. **Jackson C, Browell D, Gautrey H, *et al.*** Clinical significance of HER-2 splice variants in breast cancer progression and drug resistance. *Int J Cell Biol*. 2013; 2013: 973584.
57. **Coley HM.** Mechanisms and strategies to overcome chemotherapy resistance in metastatic breast cancer. *Cancer Treat Rev*. 2008; 34: 378–90.
58. **Fang L, Barekati Z, Zhang B, *et al.*** Targeted therapy in breast cancer: what's new. *Swiss Med Wkly*. 2011; 141: w13231.
59. **De Mattos-Arruda L, Cortes J.** Advances in first-line treatment for patients with HER-2+ metastatic breast cancer. *Oncologist*. 2012; 17: 631–44.
60. **Thery J-C, Spano J-P, Azria D, *et al.*** Resistance to human epidermal growth factor receptor type 2-targeted therapies. *Eur J Cancer*. 2014; 50: 892–901.
61. **Marchini C, Gabrielli F, Iezzi M, *et al.*** The human splice variant Delta16HER2 induces

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

rapid tumor onset in a reporter transgenic mouse. *PLoS ONE*. 2011; 6: e18727.

62. **Castiglioni F, Tagliabue E, Campiglio M, *et al.*** Role of exon-16-deleted HER2 in breast carcinomas. *Endocr Relat Cancer*. 2006; 13: 221–32.
63. **Parra-Palau JL, Pedersen K, Peg V, *et al.*** A major role of p95/611-CTF, a carboxy-terminal fragment of HER2, in the down-modulation of the estrogen receptor in HER2-positive breast cancers. *Cancer Res*. 2010; 70: 8537–46.
64. **Sperinde J, Jin X, Banerjee J, *et al.*** Quantitation of p95HER2 in paraffin sections by using a p95-specific antibody and correlation with outcome in a cohort of trastuzumab-treated breast cancer patients. *Clin Cancer Res*. 2010; 16: 4226–35.
65. **Arribas J, Baselga J, Pedersen K, *et al.*** p95HER2 and breast cancer. *Cancer Res*. 2011; 71: 1515–9.
66. **Tural D, Akar E, Mutlu H, *et al.*** P95 HER2 fragments and breast cancer outcome. *Expert Rev Anticancer Ther*. 2014; 14: 1089–96.
67. **Pedersen K, Angelini P-D, Laos S, *et al.*** A naturally occurring HER2 carboxy-terminal fragment promotes mammary tumor growth and metastasis. *Mol Cell Biol*. 2009; 29: 3319–31.
68. **Sáez R, Molina MA, Ramsey EE, *et al.*** p95HER-2 predicts worse outcome in patients with HER-2-positive breast cancer. *Clin Cancer Res*. 2006; 12: 424–31.
69. **Duchnowska R, Sperinde J, Chenna A, *et al.*** Quantitative measurements of tumoral p95HER2 protein expression in metastatic breast cancer patients treated with trastuzumab: independent validation of the p95HER2 clinical cutoff. *Clin Cancer Res*. 2014; 20: 2805–13.
70. **Andrade de Mello R, de Vasconcelos A, Ribeiro RA, *et al.*** Insight into p95HER2 in breast cancer: molecular mechanisms and targeted therapies. *Recent Pat DNA Gene Seq*. 2012; 6: 56–63.
71. **Azios NG, Romero FJ, Denton MC, *et al.*** Expression of herstatin, an autoinhibitor of HER-2/neu, inhibits transactivation of HER-3 by HER-2 and blocks EGF activation of the EGF receptor. *Oncogene*. 2001; 20: 5199–209.
72. **AbdRaboh NR, Shehata HH, Ahmed MB, *et al.*** HER1 R497K and HER2 I655V polymorphisms are linked to development of breast cancer. *Dis Markers*. 2013; 34: 407–17.
73. **Millikan RC, Hummer AJ, Wolff MS, *et al.*** HER2 codon 655 polymorphism and breast cancer: results from kin-cohort and case–control analyses. *Breast Cancer Res Treat*. 2005; 89: 309–12.
74. **Xie D, Shu X-O, Deng Z, *et al.*** Population-based, case-control study of HER2 genetic polymorphism and breast cancer risk. *J Natl Cancer Inst*. 2000; 92: 412–7.
75. **Lu S, Wang Z, Liu H, *et al.*** HER2 Ile655Val polymorphism contributes to breast cancer risk: evidence from 27 case-control studies. *Breast Cancer Res Treat*. 2010; 124: 771–8.
76. **Ma Y, Yang J, Zhang P, *et al.*** Lack of association between HER2 codon 655 polymorphism and breast cancer susceptibility: meta-analysis of 22 studies involving 19,341 subjects. *Breast Cancer Res Treat*. 2011; 125: 237–41.
77. **Chen W, Yang H, Tang WR, *et al.*** Updated meta-analysis on HER2 polymorphisms and risk of breast cancer: evidence from 32 studies. *Asian Pac J Cancer Prev*. 2014; 15: 9643–7.
78. **Rutter JL, Chatterjee N, Wacholder S, *et al.*** The HER2 I655V polymorphism and breast cancer risk in ashkenazim. *Epidemiology*. 2003; 14: 694–700.
79. **Millikan R, Eaton A, Worley K, *et al.*** HER2 codon 655 polymorphism and risk of breast cancer in African Americans and whites. *Breast Cancer Res Treat*. 2003; 79: 355–64.
80. **Kruszyna L, Lianeri M, Roszak A, *et al.*** HER2 codon 655 polymorphism is associated with advanced uterine cervical carcinoma. *Clin Biochem*. 2010; 43: 545–8.
81. **Frank B, Hemminki K, Wirtenberger M, *et al.*** The rare ERBB2 variant Ile654Val is associated with an increased familial breast cancer risk. *Carcinogenesis*. 2005; 26: 643–7.
82. **Tai W, Mahato R, Cheng K.** The role of HER2 in cancer therapy and targeted drug delivery. *J Controlled Release*. 2010; 146: 264–75.
83. **Kümler I, Tuxen MK, Nielsen DL.** A systematic review of dual targeting in HER2-positive breast cancer. *Cancer Treat Rev*. 2014; 40: 259–70.
84. **GrausPorta D, Beerli RR, Daly JM, *et al.*** ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. *EMBO J*. 1997; 16: 1647–55.
85. **Alimandi M, Romano A, Curia MC, *et al.*** Cooperative signaling of Erbb3 and Erbb2 in neoplastic transformation and human mammary carcinomas. *Oncogene*. 1995; 10: 1813–21.
86. **Yarden Y, Sliwkowski MX.** Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol*. 2001; 2: 127–37.
87. **Holbro T, Beerli RR, Maurer F, *et al.*** The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation. *Proc Natl Acad Sci USA*. 2003; 100: 8933–8.
88. **Citri A, Yarden Y.** EGF-ERBB signalling: towards the systems level. *Nat Rev Mol Cell Biol*. 2006; 7: 505–16.
89. **Slattery ML, John EM, Stern MC, *et al.*** Associations with growth factor genes (FGF1, FGF2, PDGFB, FGFR2, NRG2, EGF, ERBB2) with breast cancer risk and survival: the Breast Cancer Health Disparities Study. *Breast Cancer Res Treat*. 2013; 140: 587–601.
90. **Slamon DJ, Clark GM, Wong SG, *et al.*** Human-breast cancer - correlation of relapse and survival with amplification of the her-2 neu oncogene. *Science*. 1987; 235: 177–82.
91. **Nielsen DL, Andersson M, Kamby C.** HER2-targeted therapy in breast cancer. Monoclonal antibodies and tyrosine kinase inhibitors. *Cancer Treat Rev*. 2009; 35: 121–36.
92. **Chen FL, Xia W, Spector NL.** Acquired resistance to small molecule ErbB2 tyrosine kinase inhibitors. *Clin Cancer Res*. 2008; 14: 6730–4.
93. **Spector NL, Blackwell KL.** Understanding the mechanisms behind trastuzumab therapy for human epidermal growth factor receptor 2–positive breast cancer. *J Clin Oncol*. 2009; 27: 5838–47.
94. **Tortora G.** Mechanisms of resistance to HER2 target therapy. *J Natl Cancer Inst Monogr*. 2011; 2011: 95–8.
95. **Rexer BN, Arteaga CL.** Intrinsic and acquired resistance to HER2-targeted therapies in HER2 gene-amplified breast cancer: mechanisms and clinical implications. *Crit Rev Oncog*. 2012; 17: 1–16.
96. **Wu JY, Yu CJ, Chang YC, *et al.*** Effectiveness of tyrosine kinase inhibitors on "uncommon" epidermal growth factor receptor mutations of unknown clinical significance in non-small cell lung cancer. *Clin Cancer Res*. 2011; 17: 3812–21.
97. **Rexer BN, Ghosh R, Narasanna A, *et al.*** Human breast cancer cells harboring a gatekeeper T798M mutation in HER2 overexpress EGFR ligands and are sensitive to dual inhibition of EGFR and HER2. *Clin Cancer Res*. 2013; 19: 5390–401.
98. **Pao W, Miller VA, Politi KA, *et al.*** Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med*. 2005; 2: 225–35.

99. **Shah NP, Nicoll JM, Nagar B, *et al.*** Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell*. 2002; 2: 117–25.
100. **Antonescu CR, Besmer P, Guo TH, *et al.*** Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. *Clin Cancer Res*. 2005; 11: 4182–90.
101. **Trowe T, Boukouvala S, Calkins K, *et al.*** EXEL-7647 inhibits mutant forms of ErbB2 associated with lapatinib resistance and neoplastic transformation. *Clin Cancer Res*. 2008; 14: 2465–75.
102. **Li G, Wang X, Hibshoosh H, *et al.*** Modulation of ErbB2 blockade in ErbB2-positive cancers: the role of ErbB2 mutations and PHLDA1. *PLoS ONE*. 2014; 9: e106349.
103. **Kancha RK, von Bubnoff N, Bartosch N, *et al.*** Differential sensitivity of ERBB2 kinase domain mutations towards lapatinib. *PLoS ONE*. 2011; 6: e26760.
104. **Piechocki MP, Yoo GH, Dibbley SK, *et al.*** Breast cancer expressing the activated HER2/neu is sensitive to gefitinib *in vitro* and *in vivo* and acquires resistance through a novel point mutation in the HER2/neu. *Cancer Res*. 2007; 67: 6825–43.
105. **Wang SE, Yu Y, Criswell TL, *et al.*** Oncogenic mutations regulate tumor microenvironment through induction of growth factors and angiogenic mediators. *Oncogene*. 2010; 29: 3335–48.
106. **Falchook GS, Janku F, Tsao AS, *et al.*** Non-small-cell lung cancer with HER2 exon 20 mutation regression with dual HER2 inhibition and anti-VEGF combination treatment. *J Thorac Oncol*. 2013; 8: E19–20.
107. **Perera SA, Li D, Shimamura T, *et al.*** HER2YVMA drives rapid development of adenosquamous lung tumors in mice that are sensitive to BIBW2992 and rapamycin combination therapy. *Proc Natl Acad Sci USA*. 2009; 106: 474–9.
108. **Mazieres J, Peters S, Lepage B, *et al.*** Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives. *J Clin Oncol*. 2013; 31: 1997–U307.
109. **Cappuzzo F, Bemis L, Varella-Garcia M.** HER2 mutation and response to trastuzumab therapy in non-small-cell lung cancer. *N Engl J Med*. 2006; 354: 2619–21.
110. **Endo Y, Dong Y, Yoshimoto N, *et al.*** HER2 mutation status in Japanese HER2-negative breast cancer patients. *Jpn J Clin Oncol*. 2014; 44: 619–23.