Chapter 9
Molecular Pathology of HER Family of Oncogenes in Breast Cancer: HER-2 Evaluation and Role in Targeted Therapy

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Background

Currently, there are four different members in the epidermal growth factor receptor (EGFR) family. These consist of the erbB lineage of proteins and include erbB1 (EGFR), erbB2 (HER2), erbB3, and erbB4. Each of these molecules consists of an extracellular domain, a single hydrophobic transmembrane segment, an intracellular portion with a juxtamembrane segment, a protein kinase domain, and a carboxy terminal tail [1–3].

The *h*uman *e*pidermal growth factor *r*eceptor-*2* (*HER2* or *ERBB2*) gene product is a transmembrane growth factor receptor, which is normally expressed in secretory epithelia. It is involved in the cellular signaling that regulates growth and development [3–5]. Other HER (ErbB) proteins can preferentially heterodimerize with HER2, which leads to phosphorylation of the tyrosine residues and activation of downstream effectors such as mitogen activating protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K), and signal transducer and activator of transcription (STAT). Depending on the particular signal cascades triggered, HER2 can be involved in different biological processes, including cell survival, proliferation, differentiation, invasion, adhesion, migration, and angiogenesis, as well as malignant transformation (Fig. 9.1) [6–8].

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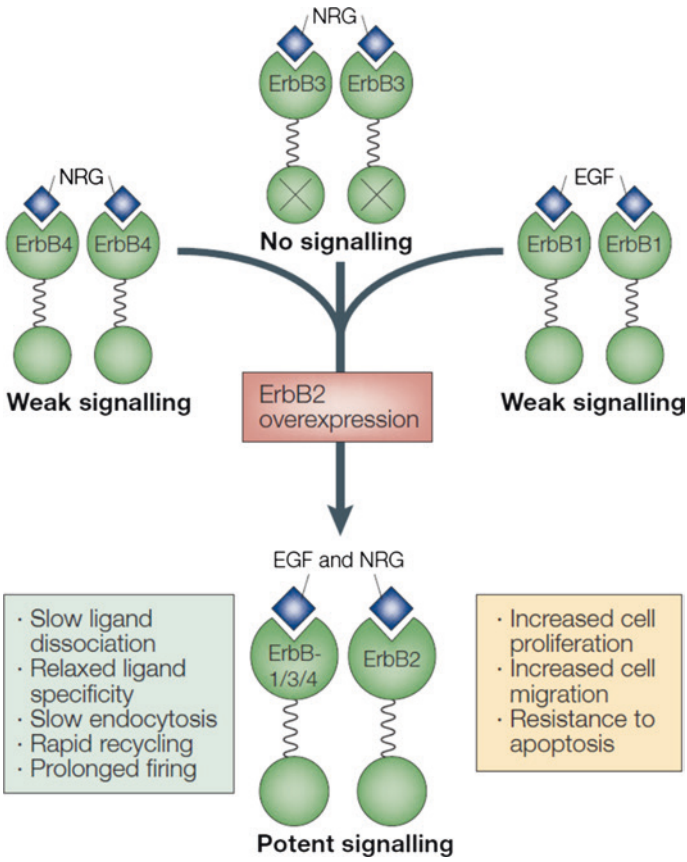

Fig. 9.1 Signaling by ErbB homodimers in comparison with ErbB2-containing heterodimers. Receptors are shown as two lobes connected by a transmembrane stretch. Binding of a ligand (EGF-like or NRG) to the extracellular lobe of ErbB1, ErbB3 (*note* inactive kinase, marked by a cross), or ErbB4 induces homodimer formation. Unlike homodimers, which are either inactive (ErbB3 homodimers) or signal only weakly, ErbB2-containing heterodimers have attributes that prolong and enhance downstream signaling (*green box*) and their outputs (*yellow box*). *NRG* Neuregulin, *EGF* Epidermal Growth Factor. With permission from [4]. Copyright Nature Publishing Group 2001

Biology of HER2

HER2 protein is expressed at low levels in normal epithelial cells [9]. *HER2* amplification and/or overexpression, however, is often observed in several cancers of epithelial origin, such as breast, colorectal, ovarian, pancreatic, and renal cell carcinomas [9, 10]. Studies using erbB2-deficient mouse models have shown lethal neural and cardiac defects during embryonic development [3, 11]. Over the past 20 years many mouse models have been developed to study the role of *HER2*

gene expression in breast cancer. These studies have shown that the erbB2 receptor can have a causal role in the development of breast carcinoma [12, 13].

The erbB-receptor family plays a crucial role in cell lineage differentiation into many tissue types, including the epithelial–mesenchymal transformation in epithelial tissues [14]. Although no ligand has been identified for erbB2, the receptor is recruited into heterodimers with other erbB receptors and this process increases ligand binding affinity of other erbB-receptor family members. Among erbB-family members, HER2 is the favored receptor for heterodimerization [6, 15].

Several mechanisms have been proposed to explain the role of erbB2 in oncogenesis. For instance, overexpression of erbB2 on the cell membrane may lead to increased heterodimerization with the kinase-defective erbB3 (HER3). These heterodimers may undergo a conformational change into the ligand-active state leading to weak, but prolonged activation of the receptor. Alternatively, spontaneous erbB2 homodimers may be formed upon overexpression of the protein with subsequent activation of the receptor tyrosine kinase [4, 16–18].

HER2 in Clinical Setting

HER2 overexpression can be seen in a number of tumors, including, but not limited to, breast, gastroesophageal, endometrial, lung, ovarian, bladder, and pancreatic carcinomas [17, 19–27]. *HER2* gene amplification is the most common mechanism driving HER2 protein overexpression. This mechanism is observed in 15−20 % of breast and gastroesophageal carcinomas and at lower rates in other carcinomas [21, 24, 26]. In normal breast tissue, the ductal epithelial cells display an average of 80,000–100,000 HER2 receptors on the cell surface, whereas breast carcinoma cells can show 500,000 to 1,000,000 receptors on their surface [28–31].

Overexpression of HER2 receptor in breast cancer leads to increased homodimerization (HER2:HER2) and heterodimerization (e.g., HER2:HER3) of the receptors, which initiates a strong pro-tumorigenic signaling cascade [4].

HER2 gene amplification has been associated with a more aggressive clinical course.

In addition, *HER2* gene amplification in breast carcinoma correlates with lymph node metastasis, negative hormone receptor status, high nuclear grade, and high proliferation index, such as high Ki67 positivity or increased mitotic activity [31–37].

Current evidence suggests that HER2 receptor overexpression can serve as a negative prognostic indicator [38]. HER2 protein overexpression has consistently been shown to act as an independent marker of poor prognosis in patients with lymph node-positive disease. Interestingly, this feature is often found in concert with other poor prognostic factors, such as large tumor size, higher histologic grade, or positive nodal status [29, 32, 38, 39].

Therapies Targeting HER2

HER2 gene amplification represents the underlying molecular event for the vast majority of HER2-driven breast cancers [40–44]. Since HER2 receptor plays a role in biological and clinical behavior of breast cancers, targeting this receptor in breast carcinomas with HER2 overexpression has been an attractive therapeutic approach. HER2 was the first molecule to be targeted with a novel humanized monoclonal antibody [45].

In 1998, the U.S. Food and Drug Administration (FDA) approved trastuzumab (Genentech, Inc., San Francisco, California), a humanized monoclonal antibody that targets the extracellular portion of the HER2 receptor. Clinical trials with trastuzumab showed that this treatment improves survival, response rates, and time to progression when used alone or in combination with chemotherapy [46–49]. Although approved for use in metastatic cancer, several prospective randomized clinical trials have also shown therapeutic benefit of trastuzumab in early stage breast cancers, by reducing the mortality rate by one-third and recurrence rate by one-half [50–55]. This therapy has been shown to be effective as a single agent or in combination with more traditional chemotherapy [56–59]. However, both clinical and in vitro studies have demonstrated that trastuzumab is only active against HER2-overexpressing (HER2 positive) tumors [49, 56, 58, 60]. There are also several reports showing that patients with relatively lower expression of HER2 protein on the cell surface derive some benefit from anti-HER2 therapy [61, 62].

Lapatinib (GlaxoSmithKline, King of Prussia, Pennsylvania), a tyrosine kinase inhibitor of HER2 and EGFR was the next therapeutic agent approved by the FDA for the treatment of HER2 positive breast cancers. Lapatinib is an ATP competitor that blocks phosphorylation of the HER2/EGFR1 tyrosine kinase domains inhibiting activation of AKT/PIK3CA and MAP kinase pathways. Lapatinib provided a significant improvement in disease-free survival of breast cancer patients after progression on trastuzumab [45, 63–65].

More recently, additional monoclonal antibody therapies have been approved for the treatment of HER2 positive metastatic breast cancer. In one instance, the original trastuzumab antibody has been conjugated to the cytotoxic agent mertansine. In one study, this antibody-drug conjugate, ado-trastuzumab emtansine (T-DM1), offered a better tolerance and improved both progression-free and overall survival when compared with the standard drug combination lapatinib–capecitabine [66]. A meta-analysis indicates that this antibody-drug conjugate is effective for HER2-positive metastatic breast cancer in patients previously treated with a variety of therapeutic agents, including trastuzumab, lapatinib, and a taxane [67, 68].

Another recently approved frontline therapy for HER2 positive metastatic breast cancer is the monoclonal antibody pertuzumab (Genentech, Inc) [69–73]. This represents a new class of monoclonal antibody that targets a different site on the HER2 molecule. Unlike trastuzumab, which binds to extracellular domain IV [74], a region that does not contribute to receptor dimerization, pertuzumab

binds to domain II and blocks dimerization of the HER2 receptor. In vitro studies have shown that pertuzumab is more effective than trastuzumab in disrupting the HER1–HER2 and HER3–HER2 dimers [75, 76]. Several clinical trials are currently underway to show efficacy and potential side effects of these therapeutic agents (NCT01966471, NCT01855828, NCT02003209). These new HER2-targeting agents have been tested in the adjuvant setting, including trials with single agent or dual antibody regimens without concomitant or sequential chemotherapy [72, 77–82]. So far, pertuzumab therapy is associated with increased progression-free survival and a strong trend toward improved overall survival [73]. All of these ongoing efforts point to the fact that accurate HER2 testing is now more critical than ever to ensure that the patients receive the correct treatment.

Resistance to HER2 Targeted Therapy

The fact that still a fraction of HER2 positive breast carcinomas treated with these targeted therapies ultimately relapse or develop a more progressive disease, suggests that there are some de novo or acquired intrinsic mechanisms of resistance to these drugs [83]. Resistance may be innate or develop during the course of HER2-targeted therapy. Some of these mechanisms include mutations in *HER2* gene itself, the use of compensatory signaling pathways and other resistance mutations affecting response to therapy (e.g., apoptosis). Mechanisms involving HER2 receptor alter the antibody binding site through alternative transcription and splicing. Compensatory signaling through other receptor or intracellular signaling pathways, such as insulin-like growth factor 1 receptor (IGF-1R), which widely bypass the HER2 receptor signaling, may also occur (Fig. 9.2). In addition, acquired mutations in *PIK3CA* or *PTEN* genes have been shown to confer resistance to trastuzumab. Finally, defects in cell cycle regulation or apoptosis, such as elevated levels of the apoptosis inhibitor survivin, as well as host factors that affect the immunomodulatory function of these drugs, may contribute to resistance [83–95].

Methods of HER2 Testing

Accurate determination of HER2 status is essential, given the significant therapeutic benefit derived from targeted therapy in HER2 positive tumors. This is underscored by the most recent American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) recommendations, which require HER2 testing of all newly diagnosed invasive breast cancers [95]. In addition, these therapeutic agents are not without complications or even serious side effects, necessitating the proper selection of patients who really benefit from them [96–98].

There are several methods that can be used to assess routine formalin-fixed paraffin-embedded (FFPE) clinical breast samples for HER2 status. These include

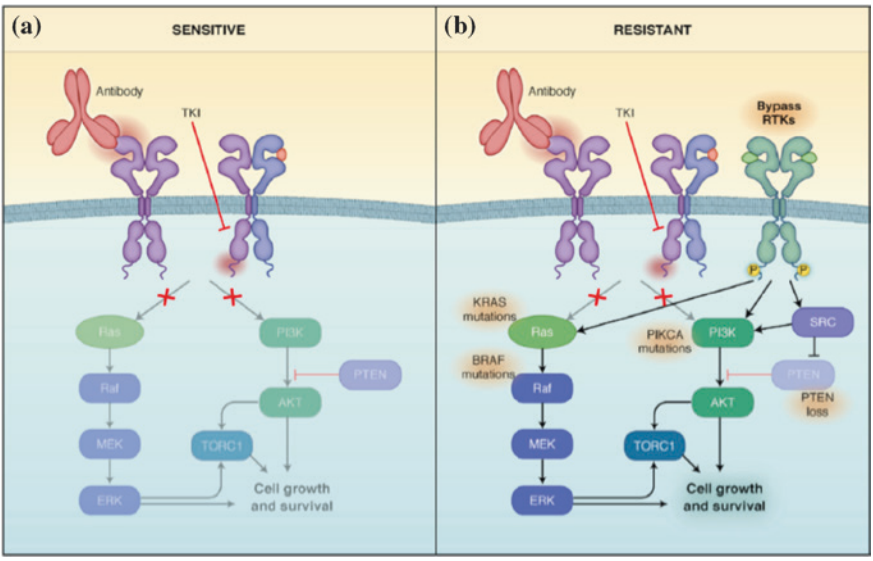

Fig. 9.2 Schematic depicting resistance to EGFR and HER2 inhibitors due to activation of bypass track signaling. **a** model of a sensitive EGFR or HER2-addicted cancer treated with an erbB small-molecule inhibitor or antibody resulting in suppression of downstream signaling. EGFR or HER2 homodimers and heterodimers are shown. **b** Model of an EGFR-mutant or *HER2*-amplified cancer with resistance due to maintenance of downstream signaling in the presence of the EGFR or HER2 inhibitors. Activation of signaling can be caused by activation of other receptor tyrosine kinases (RTKs) or mutational activation of downstream signaling. With permission from [83]. Copyright Elsevier 2014

the evaluation for HER2 protein overexpression at the tumor cell membrane by immunohistochemistry (IHC), the assessment of *HER2* gene amplification by in situ hybridization [fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH), silver in situ hybridization (SISH)], by multiplex ligation-dependent probe amplification or reverse transcription polymerase chain reaction (RT-PCR) [99–103].

Two of these methods, namely IHC and FISH, have been studied more thoroughly and gained popularity for assessing HER2 status in breast carcinomas in routine clinical practice. These methods offer several advantages. Both of these assays allow correlation between HER2 protein expression or *HER2* gene status and the morphologic features in tissue sections. Both methodologies have received FDA approval for HER2 evaluation [104, 105].

Assessment of HER2 Status by IHC

There are two FDA-approved antibodies, namely Herceptest (Dako, Carpinteria, California) and Pathway (Ventana, Tucson, Arizona), which may be used to assess HER2 protein status by IHC. These IHC systems have been reviewed in more detail elsewhere [95, 106]. A standardized scoring system for IHC studies has been developed and was most recently updated in 2013 (Fig. 9.3) [95, 104, 105].

Briefly, a positive HER2 IHC is defined by intense, complete circumferential membrane staining in >10 % of invasive tumor cells (score 3+). HER2 IHC result is negative if weak and incomplete pattern of staining is seen in ≤10 % of tumor cells (score 0/1+). In approximately 20 % of cases, an equivocal result is observed showing incomplete and/or weak to moderate circumferential staining in >10 % of the invasive tumor cells or complete and intense circumferential membrane staining is present in ≤10 % of the invasive tumor cells. All equivocal HER2 results should be reflexed to an alternative testing (i.e., FISH or CISH) on the same or another specimen, if available (Fig. 9.4) [95].

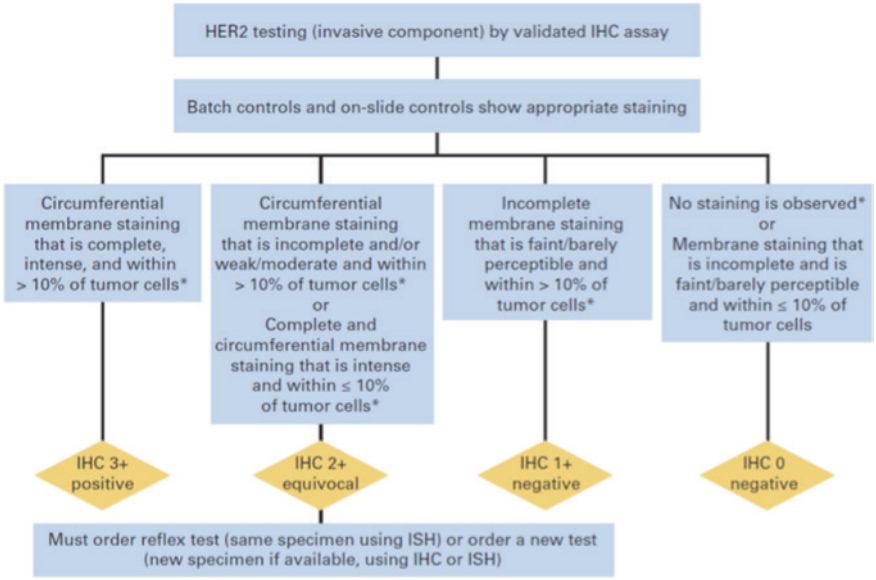

Fig. 9.3 Algorithm for evaluation of HER2 protein expression by IHC assay of the invasive component of a breast cancer specimen. *ISH* in situ hybridization. (*Asterisk*) Readily appreciated using a low-power objective and observed within a homogeneous and contiguous invasive tumor cell population. With permission from [95]. Copyright American Society of Clinical Oncology 2014

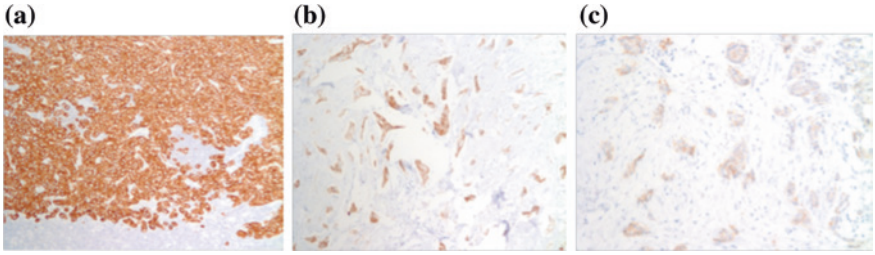

Fig. 9.4 HER2 immunohistochemistry showing 3+ (**a**); 2+ (**b**); and 1+ (**c**) staining in invasive breast carcinoma (a, b, c 100× magnification)

Assessment of HER2 Status by FISH

FISH is a molecular cytogenetic technique designed to detect specific chromosomal DNA sequences using fluorescent-labeled complementary DNA probes [106, 107]. There are three FDA-approved FISH probes manufactured by Abbott (PathVysion, Des Plaines, Illinois), Dako (HER2 FISH pharmaDx), and Ventana (INFORM, Tucson, Arizona) to assess *HER2* gene status. These FISH systems have been reviewed in more detail elsewhere [95, 106]. A standardized scoring system for FISH has been developed and was most recently updated in 2013 (Figs. 9.5 and 9.6) [95, 104, 105].

Probe sets for HER2 may include a single-color HER2 probe or dual-color probes with one sequence labeled for the *HER2* gene and the other for the centromere of chromosome 17 (CEP17). To determine amplification, an absolute *HER2* gene copy number or a ratio of *HER2* gene to CEP17 can be used. Since FISH studies have shown superior results in predicting a benefit from monoclonal antibody therapy, this approach has gained acceptance as a primary mode for *HER2* testing in breast cancer [49, 56, 59, 100, 108–112]. As *HER2* gene amplification almost always results in HER2 protein overexpression, it generally translates to 90−95 % concordance between these two methods [105]. However, 3−15 % of breast cancers may show protein overexpression without *HER2* gene amplification [63, 105, 106, 113, 114]. Recent addition of copy number to the scoring guidelines may help to identify cases with polysomy (greater than 2 copies) of chromosome 17 with HER2 protein overexpression. FISH result should be reported as positive, if dual-probe *HER2*/CEP17 ratio is ≥2.0 or an average *HER2* gene copy number ≥6.0 signals/cell. An equivocal result is defined as an average *HER2* gene copy number ≥4.0 and <6.0 signals/cell and *HER2*/CEP17 ratio <2.0. Negative result is defined as *HER2*/CEP17 ratio <2.0 and an average *HER2* gene copy number <4.0 signals/cell (Fig. 9.7) [95, 104, 108].

Although true polysomy 17 is not a common finding in breast carcinoma [115–117], in the presence of simultaneous increase in CEP17 and *HER2* gene copy number, the ratio of *HER2*/CEP17 may remain less than 2.0 and mask the true amplification of the *HER2* gene [118, 119]. In this regard, several other genes on

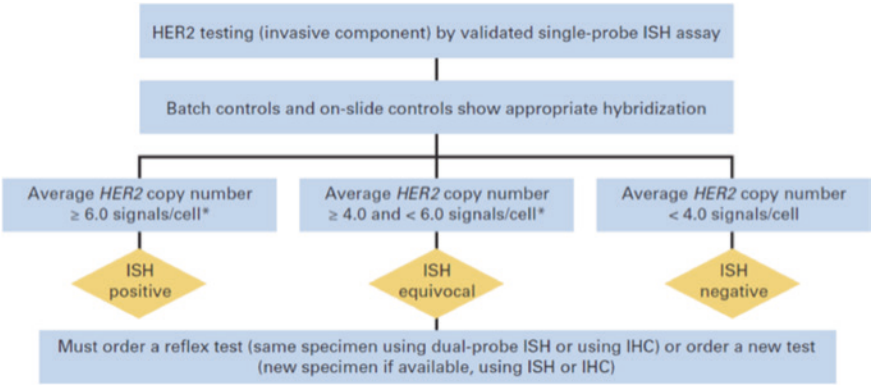

Fig. 9.5 Algorithm for evaluation of *HER2* gene amplification by ISH assay of the invasive component of a breast cancer specimen using a single-signal (*HER2* gene) assay (single-probe ISH). Amplification in a single-probe ISH assay is defined by examining the average *HER2* gene copy number. If there is a second contiguous population of cells with increased HER2 signals per cell, and this cell population consists of more than 10 % of tumor cells on the slide, a separate counting of at least 20 nonoverlapping tumor cells must also be performed within this cell population and also reported. (*Asterisk*) Observed in a homogeneous and contiguous population. With permission from [95]. Copyright American Society of Clinical Oncology 2014

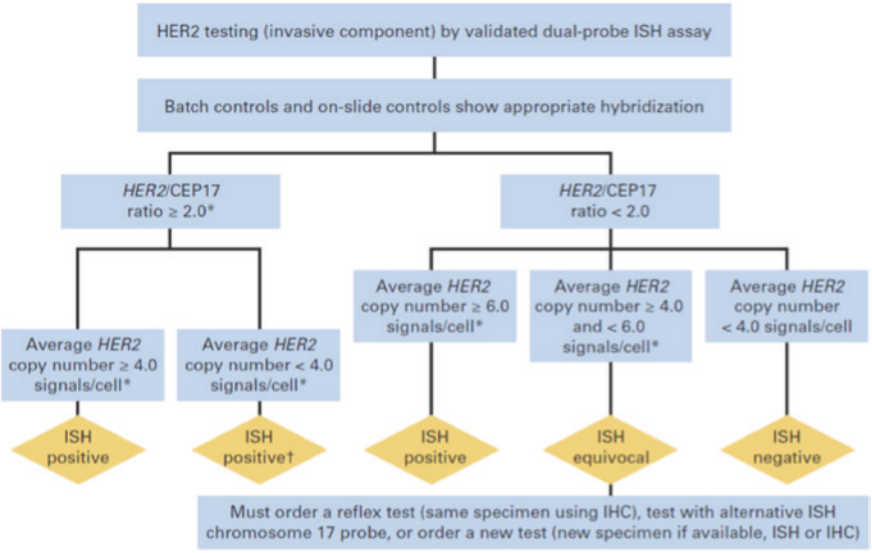

Fig. 9.6 Algorithm for evaluation of *HER2* gene amplification by ISH assay of the invasive component of a breast cancer specimen using a dual-signal (*HER2* gene) assay (dual-probe ISH). Amplification in a dual-probe ISH assay is defined by examining first the *HER2*/CEP17 ratio followed by the average *HER2* gene copy number. If there is a second contiguous population of cells with increased HER2 signals per cell, and this cell population consists of more than 10 % of tumor cells on the slide, a separate counting of at least 20 nonoverlapping tumor cells must also be performed within this cell population and also reported. CEP17, chromosome enumeration probe 17 (*Asterisk*) Observed in a homogeneous and contiguous population. With permission from [95]. Copyright American Society of Clinical Oncology 2014

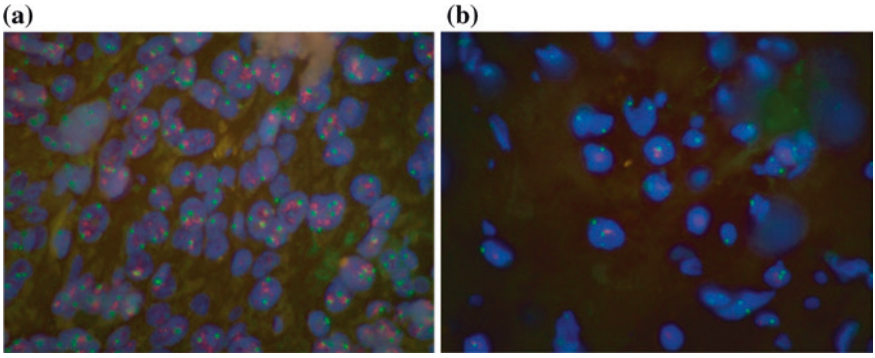

Fig. 9.7 Dual-color (*orange HER2*, *green* CEP17) FISH for *HER2* gene status on tissue sections from invasive breast carcinoma (a, b, 1000× magnification). **a** Tumor with *HER2* gene amplification; **b** tumor without *HER2* gene amplification

chromosome 17, such as *RARA, SMS,* or *TP53,* have been tested as alternative probes in determining the true *HER2* gene amplification and used successfully in different studies [120].

Brightfield In Situ Hybridization (ISH)

FISH has some disadvantages, such as the need for a dark field (fluorescence) microscope, which limits the ability to assess the conventional morphological details.

Brightfield ISH, which allows the user to assess *HER2* gene status using light microscopy, has recently been introduced as an alternative to FISH testing for the detection of *HER2* gene amplification. The current ASCO/CAP guidelines also endorse brightfield ISH methods due to high concordance with FISH and comparable clinical utility [95, 106]. Of these, chromogenic in situ hybridization (CISH) has recently been approved by the FDA. In contrast to FISH, the signals from these techniques do not fade. Therefore, the slides may be archived. Since CISH uses the brightfield microscopy, the viewer is able to easily locate the invasive tumor component to evaluate the gene status [121–124]. This method can be used to enumerate gene copy number (amplification, deletion) and chromosome translocation [125–128]. CISH similar to IHC uses enzyme-linked antibodies and chromogens to detect a hapten-labeled probe specific for the target DNA that can be applied to formalin-fixed paraffin-embedded (FFPE) tissues. Under the light microscopy the brown and red signals are visualized with better preservation of morphologic details. The interpretation of the signals may be difficult due to limitation in discriminating between discrete and overlapping signals on light microscopy [129]. However, the advantage of CISH over FISH in routine practice is that simultaneous verification of brightfield histology can be performed using CISH [130]. Although, CISH does not permit the actual determination of gene copy

number, it has been shown to correlate with FISH [131]. Silver in situ hybridization (SISH) is a novel brightfield ISH technique [130]. It is a fully automated system which uses an enzyme-linked probe to deposit silver ions on the target site that improves the efficacy and consistency of ISH and reduces the risk of error. Automated detection of chromogenic signals also allows *HER2* and CEP17 assays to be performed on consecutive tissue slides [130], making interpretation easier and resulting in a readily identifiable signals [129, 130, 132, 133]. This strategy allows *HER2* gene status to be determined in reference to chromosome 17, so that a *HER2*/CEP17 ratio can be determined using the same reported ranges as those recommended by ASCO/CAP guidelines for FISH [129, 134]. The main disadvantage of these assays is an inherent risk of sectioning through the smaller tumors, when biopsy material is used for analysis [129, 135].

Correlation of Immunohistochemistry (IHC) with Fluorescence in Situ Hybridization (FISH)

In most studies, only cases with uniform intense circumferential membrane staining (score 3+) show a good concordance with *HER2* gene amplification detected by FISH assay. This group of patients will be the most likely to benefit from HER2 monoclonal antibody therapies [49, 56, 58, 111, 136–142]. On the contrary, when there is no HER2 membrane staining or only faint and barely perceptible incomplete staining is observed (scores 0 or 1+), gene amplification studies typically demonstrate a normal *HER2* gene status and these cases are regarded as negative [137, 138, 141, 143–145]. Cases with incomplete and/or weak to moderate circumferential membranous staining (score 2+) show poor agreement with FISH results and are considered inconclusive [66, 138, 143]. In this regard, an accurate and quantitative assessment of hormone receptor (HR) results is critical, when using IHC studies to determine therapeutic targets [95, 146, 147]. It should be emphasized that a number of pre-analytical (such as tissue handling and fixation), analytical (such as reagents, antibodies, protocols), and post-analytical (reporting, quality analysis, interpretation) factors can adversely affect immune reactivity of HER2 protein [108, 148]. These are discussed in more detail in chap. 19.

Key Points

- Currently, there are four members in EGFR family of molecules. They include erbB1 (EGFR), erbB2 (HER2), erbB3 and erbB4.
- In normal states, HER2 is expressed at low levels on the surface of epithelial cells.
- HER2 protein overexpression can be seen in a number of epithelial tumors, including breast, gastroesophageal, endometrial, ovarian and lung carcinomas.

- *HER2* gene amplification as the most common mechanism for HER2 protein overexpression is seen in 15 % to 20 % of breast carcinomas.
- HER2 protein overexpression can serve as a negative prognostic factor.
- HER2 overexpression can be determined at the protein or gene levels by IHC or ISH assays.
- HER2 overexpressing breast carcinomas can be targeted by several therapeutics, including monoclonal anti-HER2 antibodies or small molecules.
- Currently trastuzumab, pertuzumab and lapatinib have been approved by FDA as targeted therapies for breast carcinomas with HER2 protein overexpression.

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