Basic Molecular Pathology in Breast Carcinoma

19

Maria Comanescu

Breast cancer is a multifactorial heterogeneous disease, reflected in a wide range of phenotypic subsets of tumors with varied degrees of aggressiveness and a significant global impact on women's health. In addition to defining the profiles of breast tumors, it is necessary to identify the individual gene and protein expression aberrations and their impact on the biology of the tumor. Molecular pathology changed the way we think about the classification of breast cancer, by no longer relying on just the histological alterations, but also on their biologic pathways. However, it should be noted that although the identification of breast cancer genes contributes to the detection of precursor lesions and prevention of invasive disease, a correlation between phenotype and genotype is necessary, as the sole assessment of gene alterations is insufficient for the identification of predictive and prognostic factors allowing the application of new and individualized cancer therapies. In conclusion, this chapter focuses on the basic molecular pathology knowledge needed in everyday routine practice.

19.1 Introduction

Diagnosis in breast cancer represents a multidisciplinary effort, combining clinical and imagistic features, histologic and immunohistochemical confirmation, and application of high-throughput technologies aiming to identify molecular targeted agents. The role of the pathologist is to apply in practice the new diagnostic tools to improve patient care (Fig. 19.1).

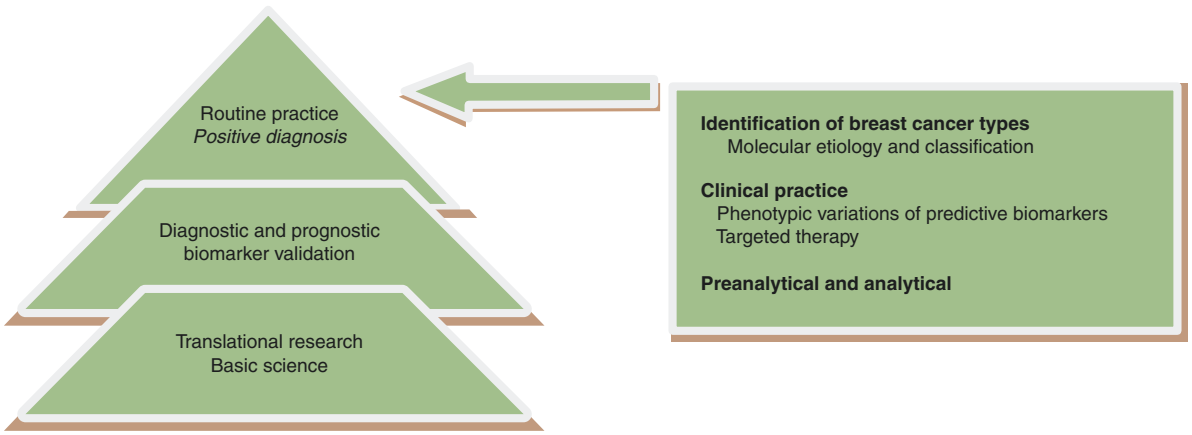

Fig. 19.1 How does molecular pathology fit into everyday practice? The ending point of all research is the development of assays that are robust and reproducible

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19.2 Identification of Breast Cancer Subtypes

Modern diagnosis is based on:

- Clinical and imagistic features
- Histological and immunohistochemical confirmation
- Identification of molecular targets for a personalized therapy (Fig. 19.2)

The molecular classification of breast carcinoma was first proposed by Perou et al. [1], who divided a set of 1753 genes into four categories based on their gene expression profile:

- Luminal-like
- Basal-like
- Her2/neu positive
- Normal-like

This classification, which first emphasized the division of all breast carcinomas into estrogen (ER)-positive and ER-negative, has been validated by multiple studies, and several subtypes have been added to the initial panel.

Immunohistochemical surrogate algorithms were created to bring this classification into practice.

Different phenotypes of breast cancer reflect different cell types. The neoplastic precursor lesions and breast carcinomas are formed by epithelial cells transformed as a result of genetic and epigenetic changes. The similarity between markers expressed by normal mammary gland cells and those identified in various tumors has resulted in the etymology used in the molecular classification of breast tumors.

As pictured in Fig. 19.3, the adult normal breast epithelium (disease-free) has alveoli lined by:

- Luminal cells (ductal and alveoli luminal cells)—basophile, ER+ and −.
- Basal cells—clear cytoplasm and oval nuclei with conspicuous nucleoli, ER-Cells that are HR receptor positive are different from the proliferating cells (ki67 positive)—the control mechanism of proliferation by ER is indirect [2].

Because cytokeratins (CK) have a constant expression during carcinogenesis, they indicate the cell of origin [3]:

- Luminal cells—low molecular weight CK - CK7, CK8, CK18, CK19
- Basal cells—high molecular weight CK - CK5/6 (Fig. 19.4), CK14, CK17 but also p63 (Fig. 19.5), SMA, CD10, S100

The present guidelines recommend the molecular classification based on several immunohistochemical markers (Fig. 19.6) [4]. Prognosis of patients with ER-positive breast carcinoma depends on the expression of proliferation-related genes [5].

"Immunohistochemistry-Based Molecular Subtyping" is used in practice due to its good correlation with gene expression assays [6], and it has clinical implications (Fig. 19.7).

The basal-like has received special attention due to its aggressive evolution and lack of targeted therapies. This subtype overlaps with the triple negative in ~80% overlap [10]. It has been characterized by the "core basal profile" [12]:

- ER negative, PR negative, HER2 negative
- CK5/6 and/or EGFR positive
- High-grade histologic features
- Pushing borders
- High-grade cytology
- Tumor-infiltrating lymphocytes

Many BRCA1 positive breast cancers are basal-like, but all basal-like cancers are not BRCA1 positive, and CK14 positivity improves the prediction of BRCA1 status [13].

This subgroup also includes several histologic subtypes that are low-grade adenoid cystic carcinoma, apocrine carcinoma, some metaplastic carcinoma variants.

Due to this heterogeneity, it has been divided in four subgroups [14]:

- Luminal androgen receptor subtype (AR positive)
- Mesenchymal subtype (stem-like and claudin low subtype)
- Basal-like immunosuppressed subtype (downregulation of immune regulating pathways)
- Basal-like immunostimulated subtype (upregulation of immune regulating pathways)

Several commercial multiparameter gene expression analysis tools have entered clinical care and are available to patients, such Oncotype DX or Mamma Print. Both of these include hormone receptor and Her2 evaluation:

- Mammaprint Fresh/ FFPE (Agendia, Irvine, CA www.agendia.com)
  - Separates tumors into two categories: high risk and low risk of recurrence
  - Identifies 70 genes by microarray (genes involved in cell cycle, invasion, metastasis, and angiogenesis) [15]
  - Requires fresh tissue as well as formalin-fixed paraffin embedded tissue
- Oncotype dx (Genomic Health, Redwood, CA, www.oncotypedx.com)
  - Separates tumors into three categories: high risk, low risk, and intermediate risk of recurrence
  - Has therapeutic implications
  - Identifies 21 genes by qRT-PCR
  - Requires formalin-fixed paraffin embedded tissue
- ADJUVANT! Online is an online algorithm to determine the benefit of chemotherapy based on clinical and pathologic data (www.adjuvantonline.com)

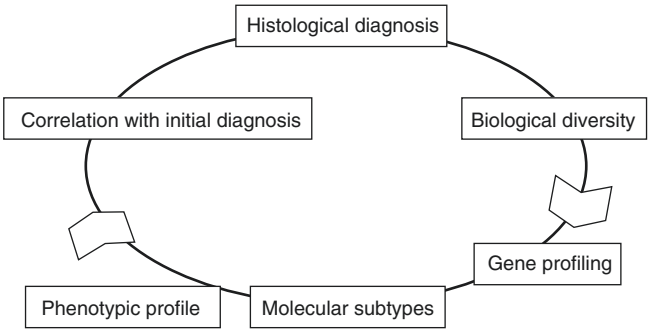

Fig. 19.2 Relationship between histological and molecular subtyping of breast carcinoma. Because all molecular information must be correlated with the clinical and histological findings, the first step is the identification of the morphologic variation of breast carcinoma. Histologically, most breast carcinomas will fall in the "invasive ductal carcinoma, not otherwise specified" type, disregarding the biological diversity. Although this initial "profiling," based on the Nottingham score, is important for therapy, there was a need for more information. The genetic study of breast cancers has brought new prognostic and predictive biomarkers into practice, as well as targeted therapies. On the other hand, molecular profiling is not available in a common pathology department

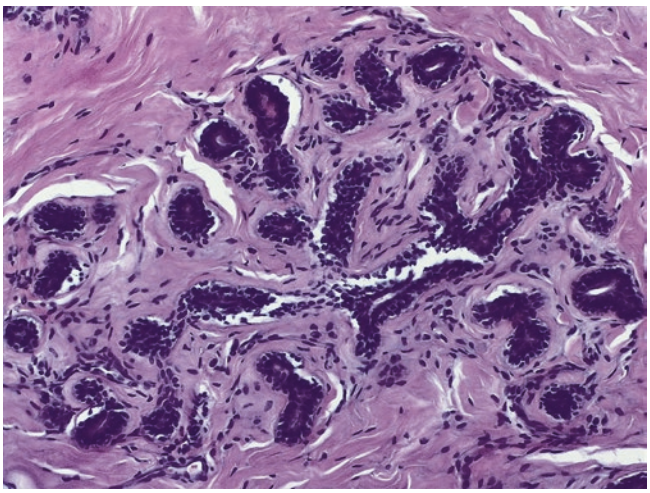

Fig. 19.3 Normal structure of the breast epithelium

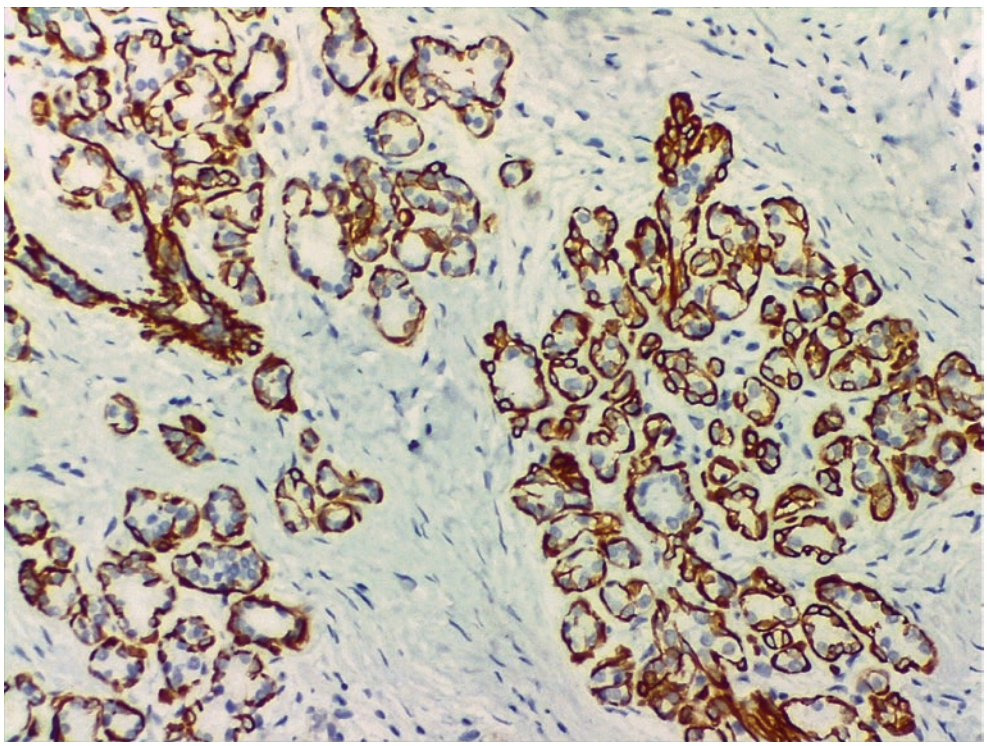

Fig. 19.4 CK5/6 positive in myoepithelial

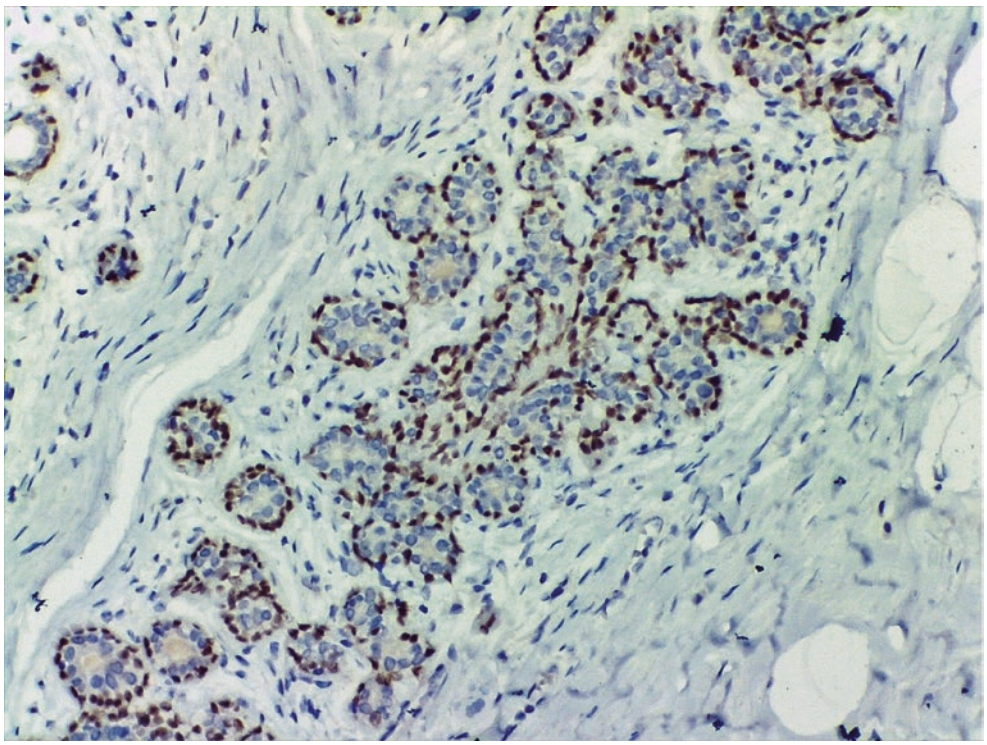

Fig. 19.5 p63 positive in myoepithelial cells

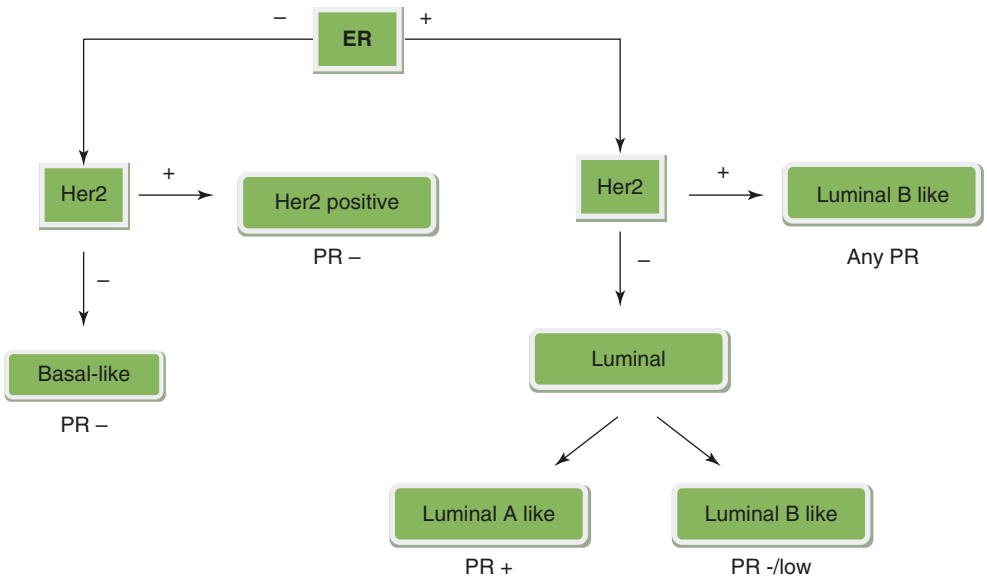

Fig. 19.6 Breast cancer "molecular" classification using immunohistochemical tests

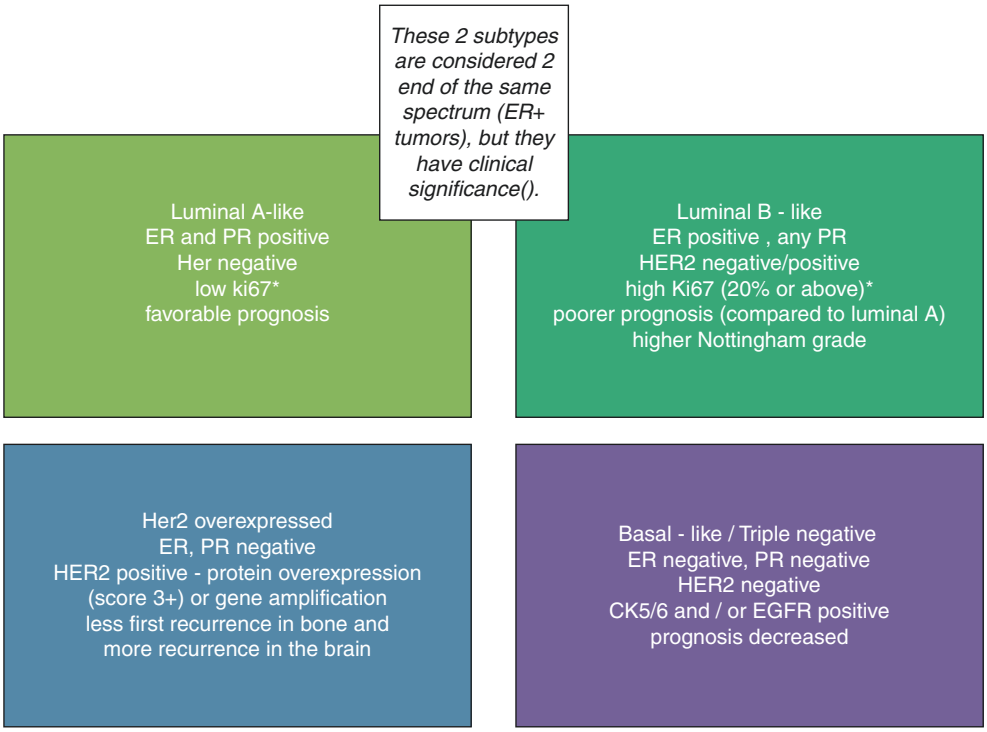

Fig. 19.7 Molecular subtypes of breast carcinomas. *ki 67—a 14% cutoff [9] and a cutoff of greater than 20% [10] per the 2013 St. Gallen Conference [7, 8, 11]

19.3 Clinical Practice

19.3.1 Phenotypical Variations of Predictive Biomarkers

ER, PR, and Her2 determination is reflex in all cases of breast carcinoma, and several combinations are encountered in clinical practice [16]:

- ER/PR+, Her2+ = ER+/PR+, Her2+; ER−/PR+, Her2+; ER+/PR−, Her2+
- ER/PR+, Her2− = ER+/PR+, Her2−; ER−/PR+, Her2−; ER+/PR−, Her2−
- ER/PR−, Her2+ = ER−/PR−, Her2+
- ER/PR−, Her2− = ER−/PR−, Her2−

19.3.1.1 Hormone Receptors

The analysis of hormone receptors, especially ER and PR, is used for a better stratification of risk and the evaluation of sensitivity to endocrine therapy (Fig. 19.8).

Evaluation of HR status is done with IHC, by estimating a percent of positive nuclei (Fig. 19.9) This interpretation is subjective and influenced by preanalytical, analytical, and postanalytical conditions.

ASCO 2013 [10] advises fixation in 10% neutral buffered formaldehyde for not less than 6 h and no more than 72 h, but recent data [17] showed that same-day diagnosis with brief fixation (60–90 min) is reliable.

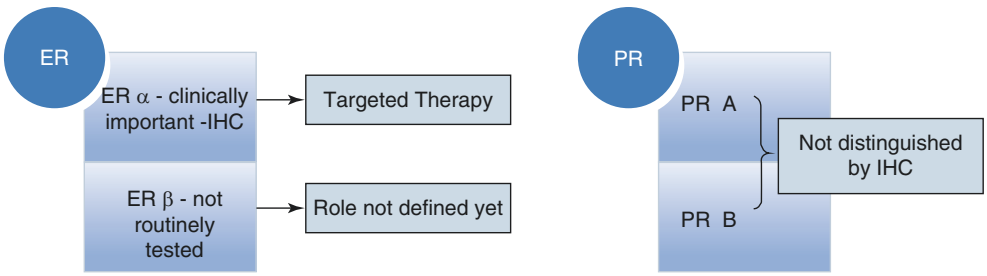

Fig. 19.8 Hormone receptors variants. ER has 2 isoforms, α and β, encoded by different genes

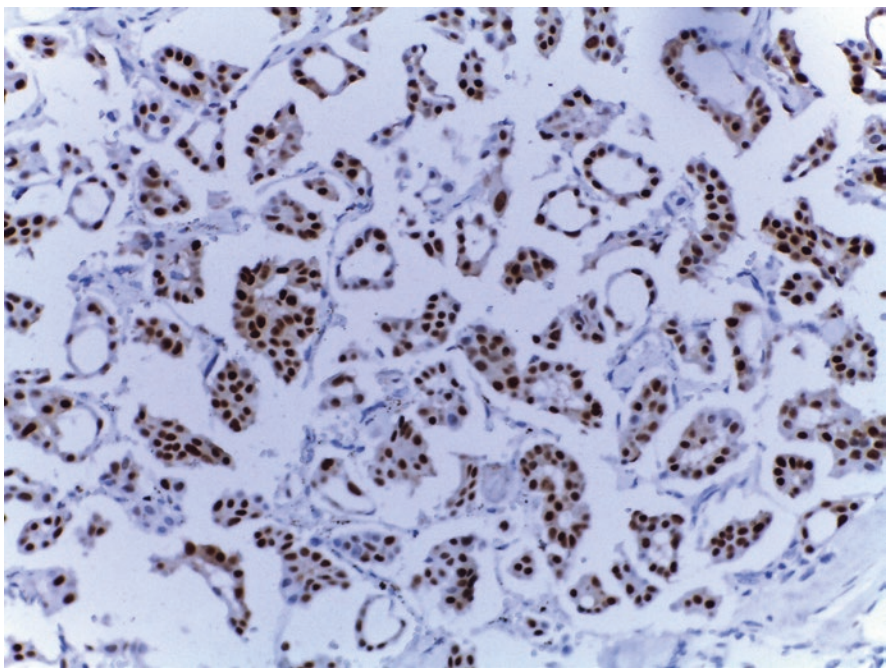

Fig. 19.9 ER receptor positive breast carcinoma

19.3.1.2 HER Receptor Family

Human epidermal growth factor receptor 2 (HER2) is a member of the epidermal growth factor receptor family, a family of glycoproteins with tyrosine kinase activity.

- Glycoproteins with tyrosine kinase activity
- Her2/c-erbB2/ is involved in 20–30% of human breast cancers [18]
- Formed by (Fig. 19.10):
  - Extracellular—ectodomain—binding activity
  - Transmembrane domain
  - Intracellular—endodomain—tyrosine kinase activity

Her2 status should be determined in all newly diagnosed cases, as well as in recurrences and metastasis. There are several available tests for determination of Her2 status:

- Immunohistochemistry (evaluation of protein expression on the surface cell) (Fig. 19.11)
- In situ hybridization (gene amplification): the gold standard
  - CISH—uses chromogens for identifying signals
  - DISH—dual stain
  - FISH—uses fluorescence

CISH detection using the commercial kit from SPoT-Light HER2 CISH kit (Zymed Laboratories Inc., USA), is based on the gene copy number:

- Non-amplified, 2–5 brown intranuclear spots/nucleus (Fig. 19.12)
- Low-level amplification, 6–10 signals/nucleus (Fig. 19.13)
- High-level amplification, >10 copies per nucleus/clusters (Fig. 19.14)

The method most frequently used in present is DISH (INFORM HER2 Dual ISH DNA Probe Cocktail -Ventana Medical Systems, USA), which has the following advantages:

- Correlation with morphology
- Signals don't fade
- Doesn't require fluorescence microscopy

Although FISH remains the gold standard, it has several disadvantages:

- Expensive
- Time-consuming
- Very specific training
- Not universally available

Interpreting ISH analysis presumes enumerating signals for the target (Her2) and the control (CEP17) (chromosome 17 centromere) and determining the ratio of Her2 gene copies to Chromosome 17/CEP17 copies/tumoral cell nucleus and average Her2 signal count per tumoral cell (Figs. 19.15, 19.16, 19.17, and 19.18).

Although ISH is the gold standard, there are still equivocal cases in HER2 testing. The recommendation of ASCO 2013 for such cases is the use of alternative FISH probes. Problems in interpretation can be due to (Fig. 19.19):

- Polysomy = extra copies of chromosome 17 leading to increased HER2 gene copy number
- CEP 17 amplification = three or more copies of chromosome 17 centromere (Fig. 19.20)

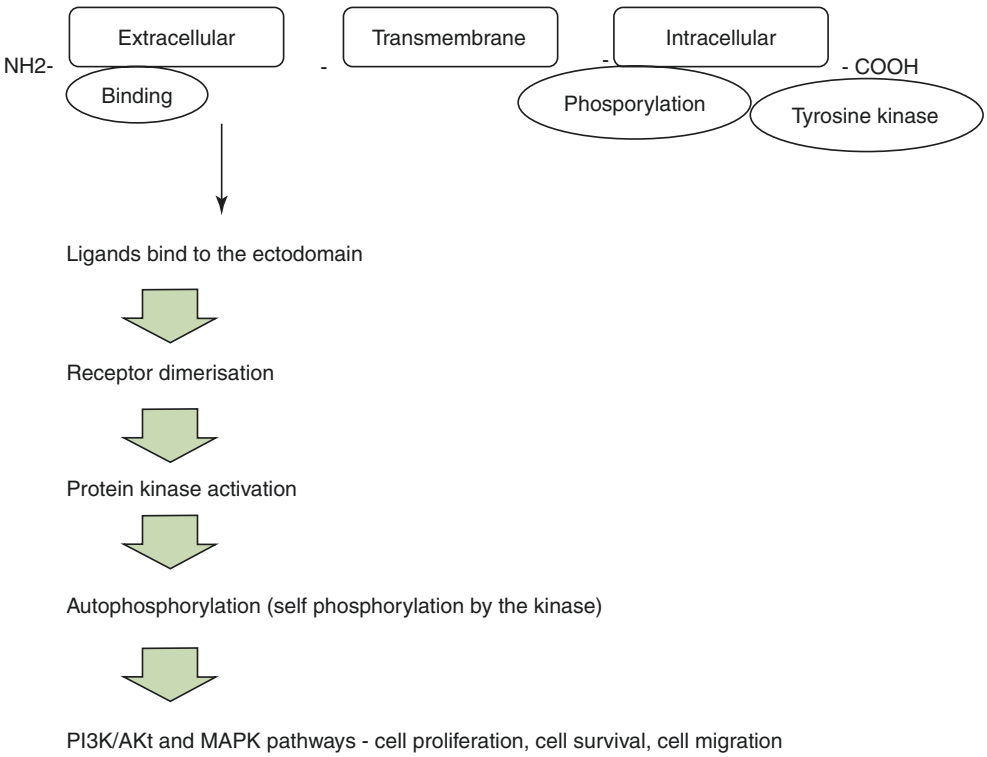

Fig. 19.10 Schematic representation of Her2 gene and mechanism of Her2 activation [19]

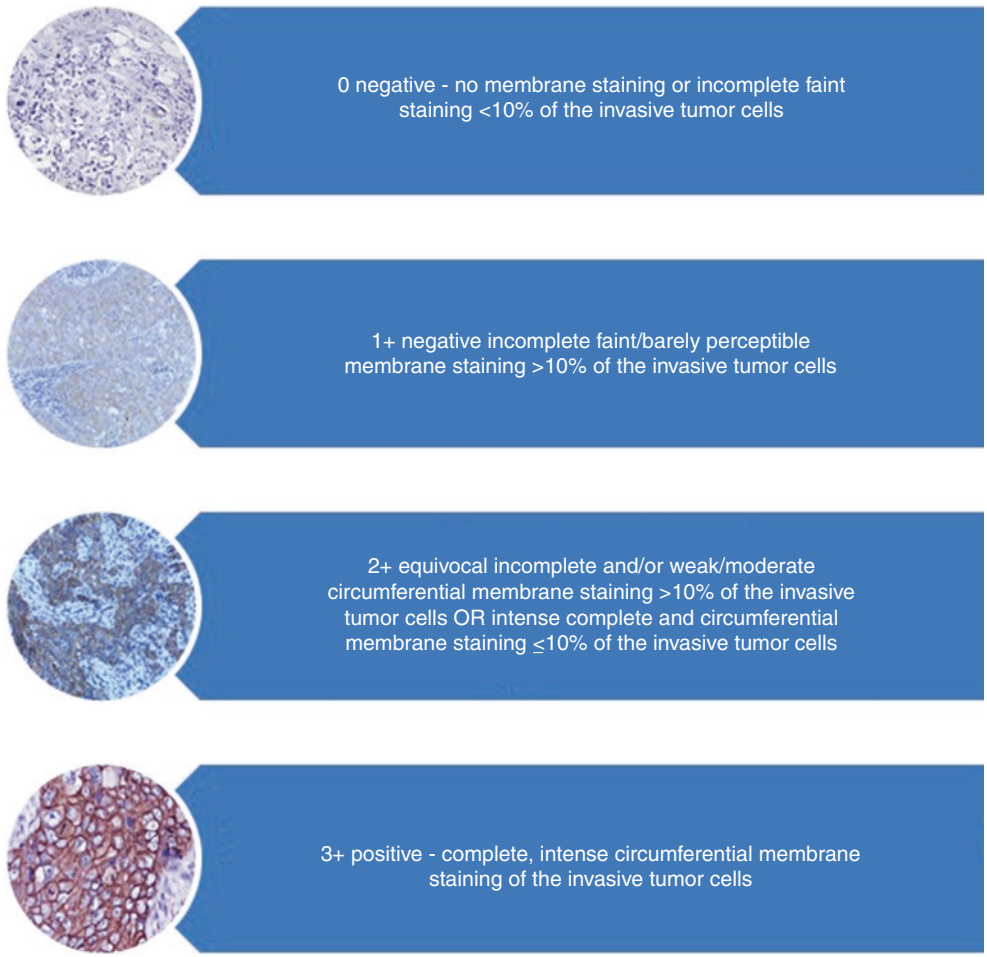

Fig. 19.11 Immunohistochemical algorithm for the evaluation of Her2 status [20]

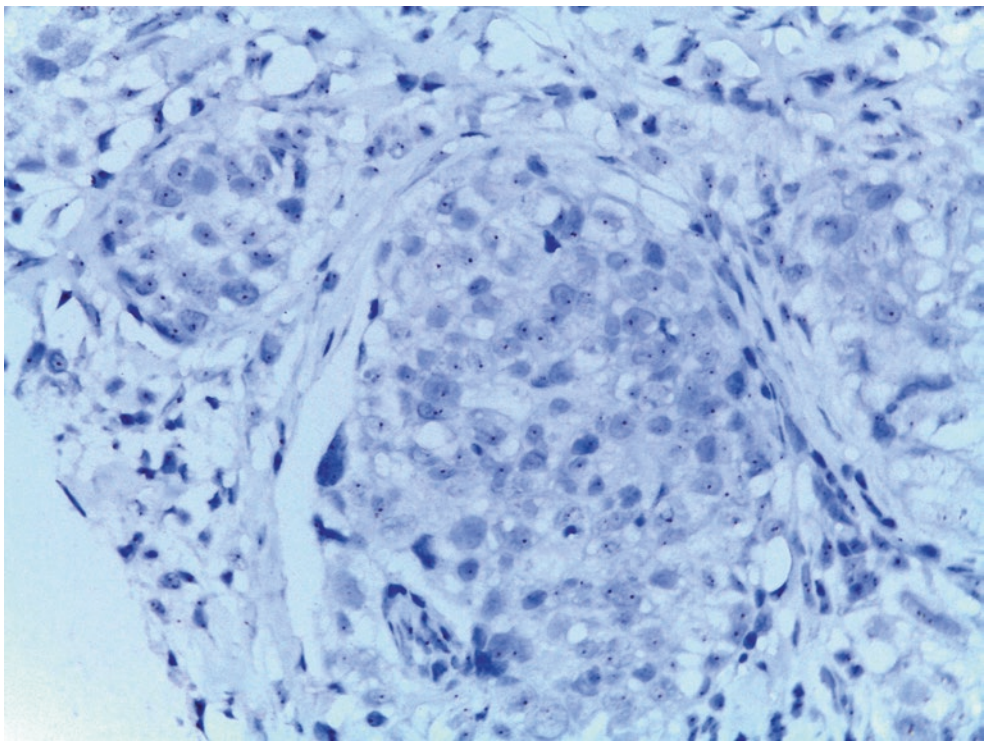

Fig. 19.12 CISH non-amplified

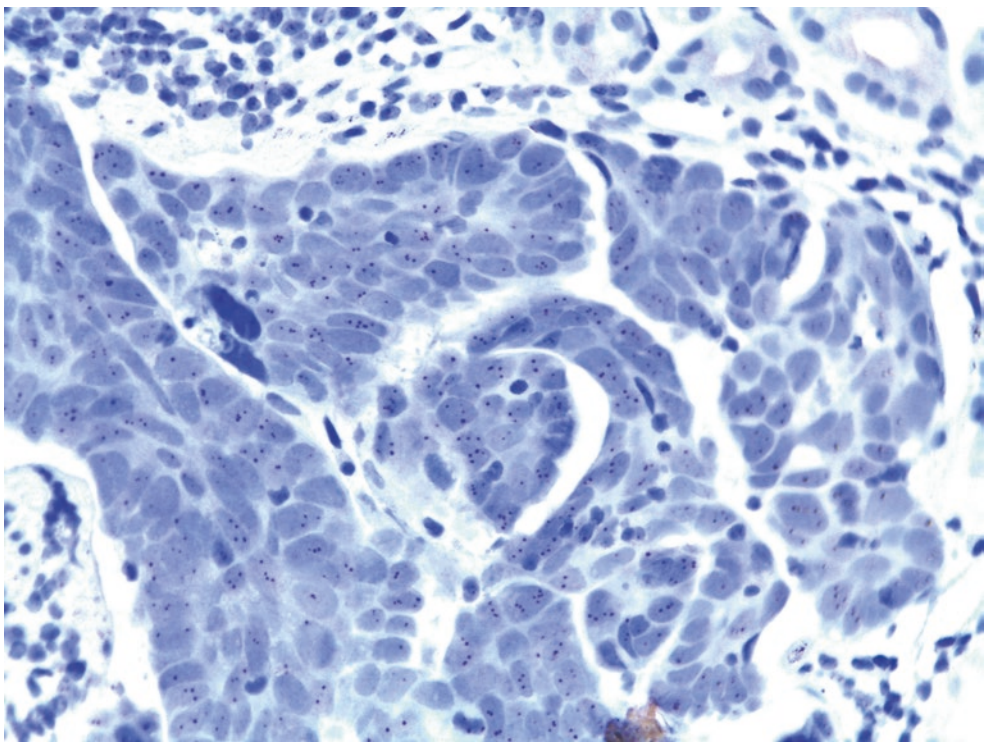

Fig. 19.13 CISH low level amplification

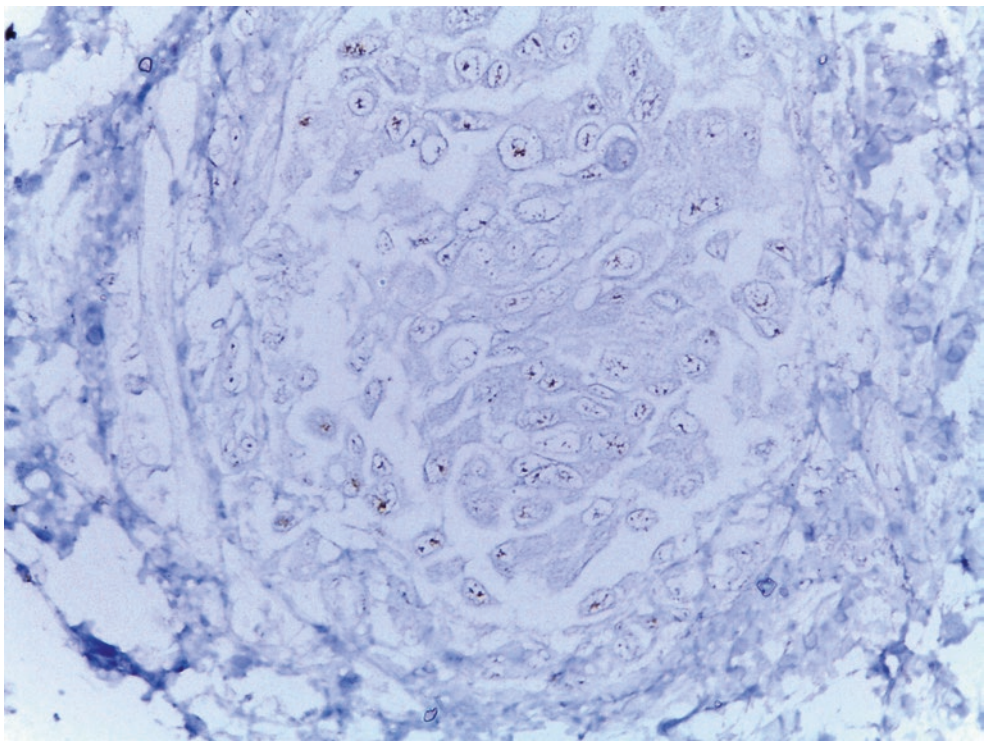

Fig. 19.14 CISH high level amplification

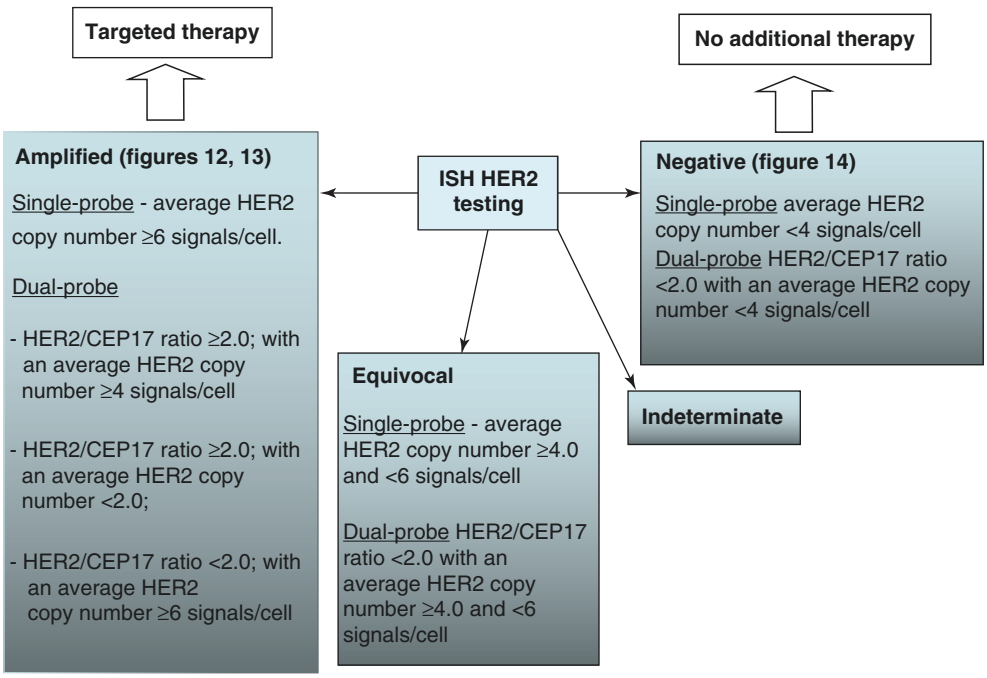

Fig. 19.15 ASCO–CAP HER2 test guideline recommendations (2013) [20]

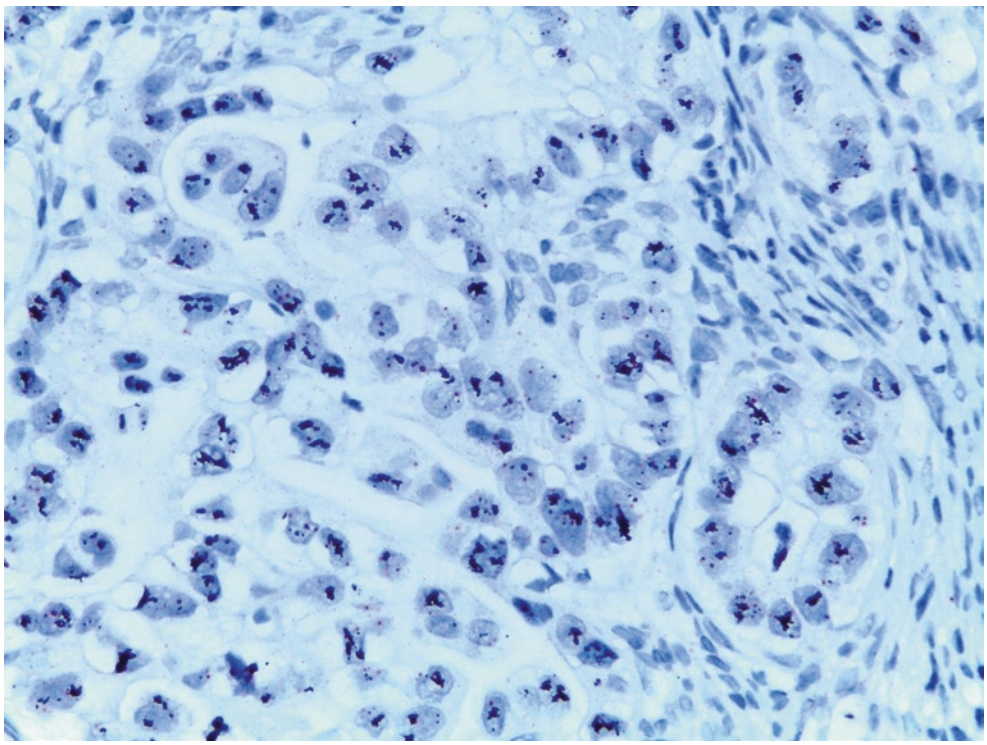

Fig. 19.16 DISH amplified

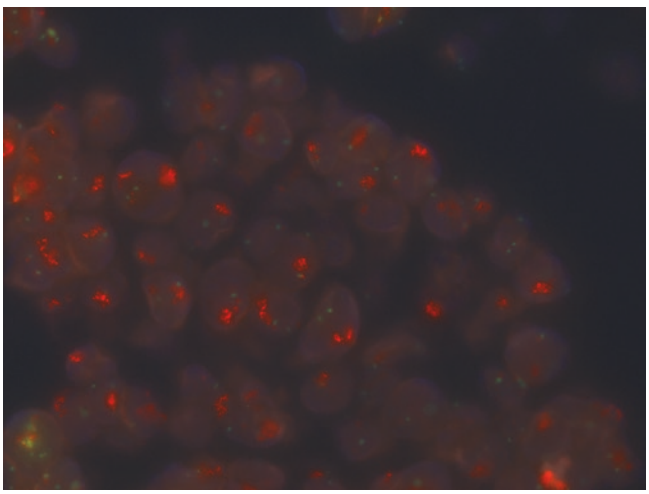

Fig. 19.17 Heterogenous breast cancer—background of Her2 amplified cells with high level amplification (red) together with isolated, intermingled nonamplified cells. Positive reaction

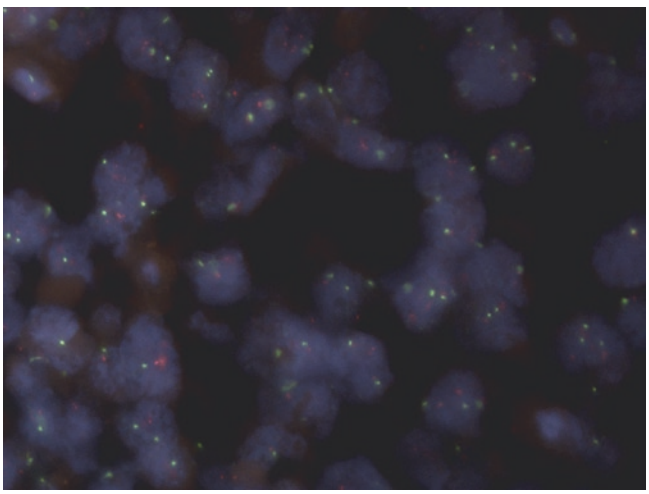

Fig. 19.18 Nonamplified tumor cells. Negative reaction

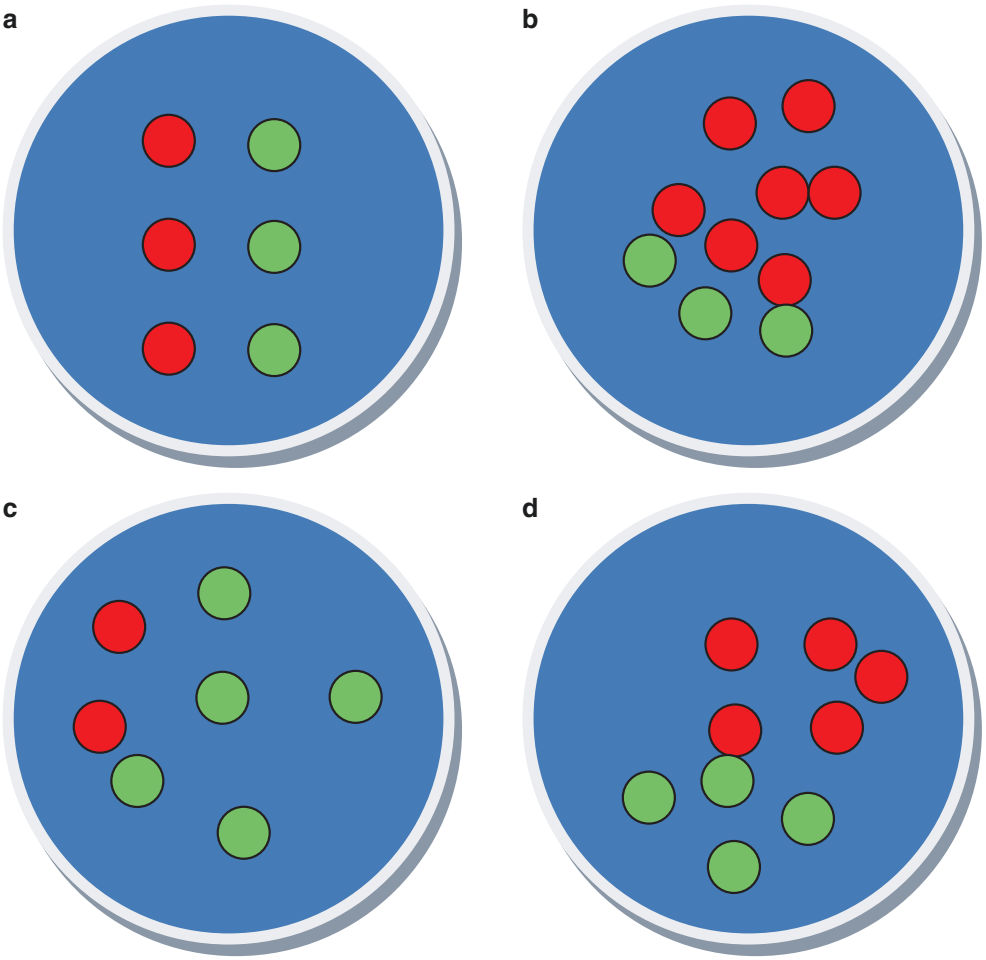

Fig. 19.19 Diagram showing polysomy and coamplification. (**a**) Polysomy. (**b**) Polysomy and Her2 amplification. (**c**) CEP 17 gain. (**d**) CEP17 gain and Her2 amplification

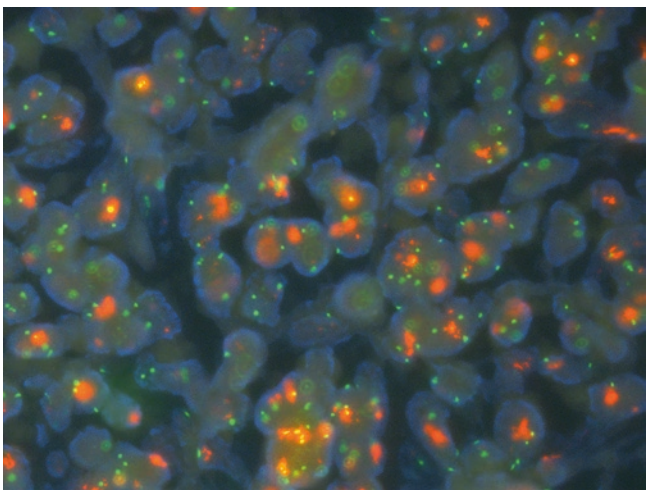

Fig. 19.20 Breast carcinoma. Her 2 amplified cells (red) and CEP 17 gain (green)

19.4 Cancer Predisposition

There are two types of genetic alterations that can initiate carcinogenesis [21]:

- Activation of protooncogenes
- Inactivation of suppressor genes

Around 10% of breast carcinomas are hereditary, tumors caused by mutations in a single high penetrance susceptibility gene, and they are histologically, phenotypically, and genotypically different from sporadic tumors.

The two-hit hypothesis explains the difference between hereditary and sporadic cancers and how mutations in suppressor genes occur [22] (Fig. 19.21).

Genetic/inherited breast cancers can be site-specific and are most commonly associated with BRCA1 or 2 mutations. They can be associated with other carcinomas, like the ones encountered in the Li-Fraumeni or Cowden syndrome.

BRCA1 and BRCA2 defects increase the lifetime risk of developing breast cancer (57% in BRCA1 mutation carriers and 49% in BRCA2 mutation carriers) [23].

The histopathological profile of hereditary tumors is different in the following ways:

- They appear in young women
- They have a higher histological grade
- They have abundant lymphocytic infiltrate
- They have pushing borders
- They are characterized by geographic necrosis
- They are usually triple negative, basal CK positive, high expression of p53
- They cluster with basal-like carcinomas

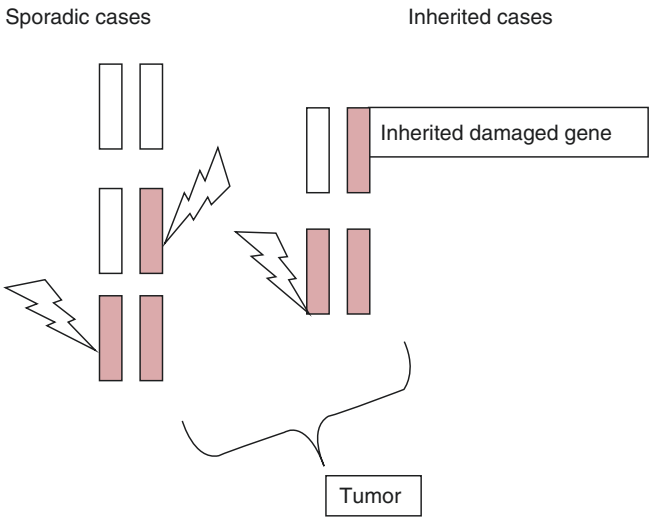

Fig. 19.21 The Knudson two-hit theory. Normally, there are two alleles of each tumor suppressor gene, and both must become inactivated in order to progress to cancer. In inherited cancers, there is already an inactivating mutation in one of the alleles

19.5 Circulating Tumor Cells

Circulating tumor cells (CTC) are migrating tumor cells that detach from the primary tumor and enter the blood stream, where they can suffer multiple transformations. Clinical detection of CTC has a prognostic relevance (Fig. 19.22).

Detection of CTC in the peripheral blood ("liquid biopsy") (Fig. 19.23) can be laborious owing to their very low concentrations, but detection has clinical importance because these cells have been demonstrated to have different genotypic profiles from the primary tumor [25].

CellSearch™ system (Veridex, LLC, Raritan, NJ, USA, www.cellsearchctc.com) is an FDA-approved method that works as an independent predictor of outcome, based on the number of CTC:

- <5 CTCs—more than 18 months survival
- ≥5 CTCs—less than 11 months survival

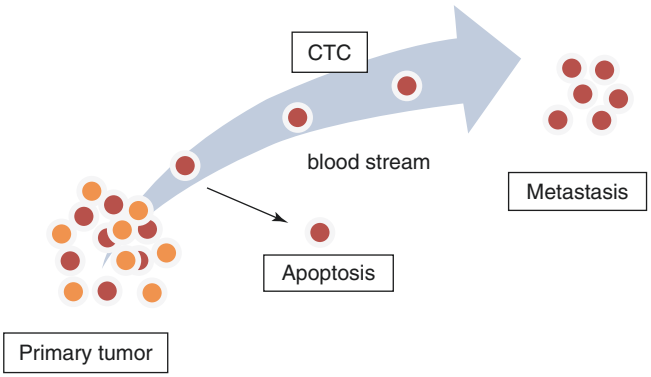

Fig. 19.22 CTC fate. CTC are released from the primary tumor in the blood flow, where they can undergo apoptosis or migrate to secondary sites (metastasis). Some CTC can become dormant [24]

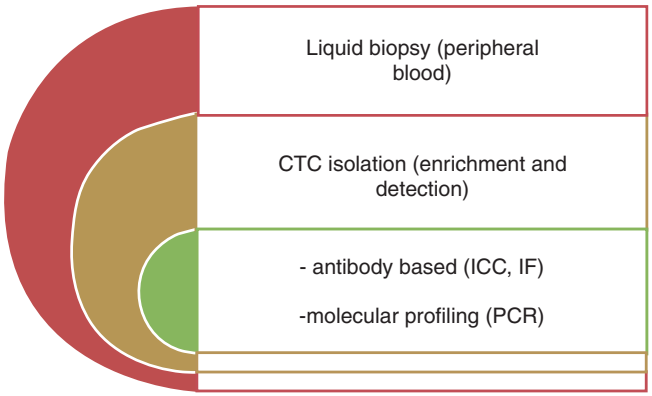

Fig. 19.23 Liquid biopsy is a noninvasive method of identifying CTC in the peripheral blood. Because of their low concentration and admixture with blood cells, special methods of isolation are needed, which generally involve labeling with cytokeratins and antibodies specific for leukocytes

19.6 Exosomes: Does the Future Lie There?

Exosomes (circulating miRNA) are small vesicles derived from cells which can cross the physiological barriers and are involved in breast cancer invasion and metastasis, drug resistance, angiogenesis and has dual effect on the immune system [26]

Conclusions

Although molecular high throughput techniques are not available in routine practice, the tissue needed for such procedures is. These tests require a proper preservation of tissues and special handling when being referred to specialized molecular laboratories (Fig. 19.24)

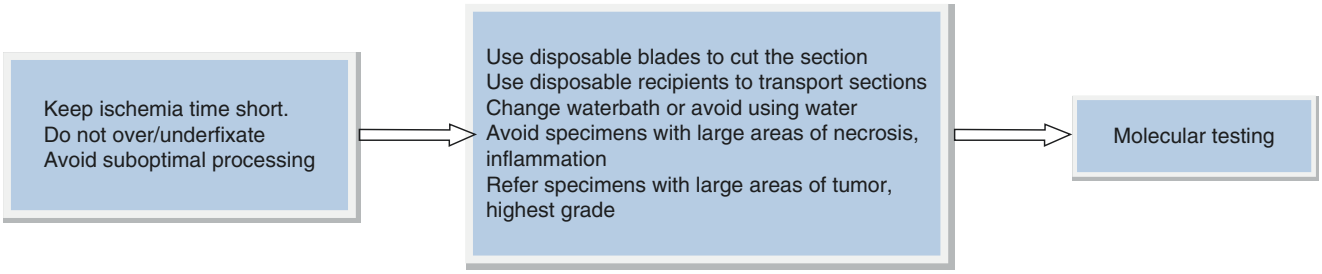

Fig. 19.24 Preparing tissue sections for molecular testing

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