Markers and Immunoprofile of Breast Tumors

10

Contents

Normal breast tissue comprises mesenchymal and epithelial components, which include luminal ductal and acinar (lobular) cells and myoepithelial/basal cells, each cell type with its characteristic immunoprofile. The immunoprofile of breast tumors depends on the origin of neoplastic cells.

10.1 Diagnostic Antibody Panel for Breast Carcinoma

10.1.1 Markers for Luminal Cells

Cytokeratin profile, E-Cadherin, GATA-3, estrogen and progesterone receptors, Mammaglobin, GCFPD-15, TRPS-1, and NY-BR-1.

M. S. A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
https://doi.org/10.1007/978-3-031-45024-2_10

10.1.2 Markers for Basal/Myoepithelial Cells

CK5/6/14, S100P, Sox-10, sm-Myosin, sm-Actin, and Calponin.

10.1.3 Therapy-Related Markers

Steroid hormone receptors (estrogen, progesterone, and androgen receptors); HER-2; NTRK; PD-L1; Trop-2; and Ki-67 [1].

10.2 Diagnostic Antibody Panel for Fibroepithelial Tumors

Cytokeratin profile, CD34, and proliferation index (Ki-67).

10.3 Diagnostic Antibody Panel for Mesenchymal Tumors

See panels of other mesenchymal tumors.

10.3.1 GATA-3

GATA-3		
Expression pattern: Nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Breast carcinoma – Transitional cell carcinoma of the urinary tract – Brenner tumor – Tumors of skin adnexa – Yolk sac tumor – Salivary gland tumors – Paraganglioma – Parathyroid adenoma/carcinoma – Clear cell papillary renal tumor	Endometrioid carcinoma, trophoblastic tumors/choriocarcinoma, cervical mesonephric carcinoma, basal cell carcinoma, mesothelioma, pancreatic ductal adenocarcinoma, colorectal adenocarcinoma, chromophobe renal cell carcinoma, bladder small cell carcinoma, neuroblastoma, pituitary adenoma (gonadotroph pituitary adenoma), pheochromocytoma, adrenal cortical carcinoma, squamous cell carcinoma of different locations, peripheral T-cell lymphoma, tumors of ceruminous glands	Luminal adult breast, terminal ducts of the parotid gland, distal renal tubules and renal pelvic and urinary bladder urothelium, prostatic basal cells and seminal vesicle epithelium, cortex and medulla of the adrenal gland, ductal epithelium of skin adnexa and salivary glands, trophoblasts (mainly intermediate), T-lymphocytes
Positive control: Normal breast tissue		

Diagnostic Approach GATA-3 (GATA-binding protein 3 to DNA sequence [A/T]GATA[A/G]), also known as endothelial transcription factor 3, is one of the six members of the GATA family of transcription factors divided into two groups. GATA-1, GATA-2, and GATA-3 are involved in the regulation of proliferation and differentiation of hematopoietic cells and the nervous system. The second group includes GATA-4, GATA-5, and GATA-6, participating in the regulation of mesoderm and endoderm, including the gastrointestinal tract, genitourinary, and respiratory system.

GATA-3 plays an essential role in the differentiation of T-lymphocytes and early erythropoiesis beside skin adnexa, breast and salivary glands, adrenal and parathyroid glands, neuronal cells, and placenta.

In diagnostic immunohistochemistry, GATA-3 is widely used as a marker for primary and metastatic breast carcinoma and transitional cell carcinoma (Figs. 10.1 and 10.2) [2, 3]. In breast carcinomas, the expression of GATA-3 strongly correlates with the expression of the estrogen receptors but lacks therapeutic and prognostic value. The expression of GATA-3 is found in up to 90% of breast carcinomas, while the lowest expression level is found in triple-negative breast carcinomas as well as metaplastic and sarcomatoid breast carcinomas (less than 70%). Only one-third of male breast carcinomas are positive for GATA-3 [4]. Generally, high expression lev-

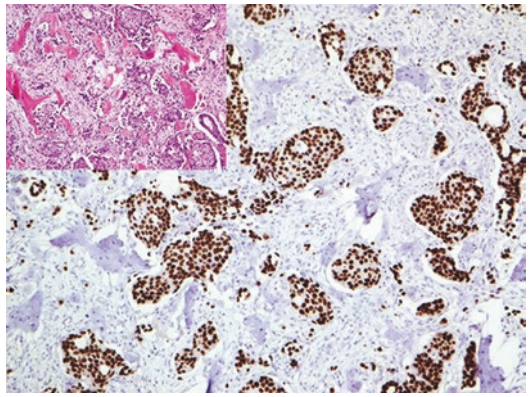

Fig. 10.1 Bone metastases of invasive ductal breast carcinoma. Tumor cells with strong nuclear GATA-3 expression

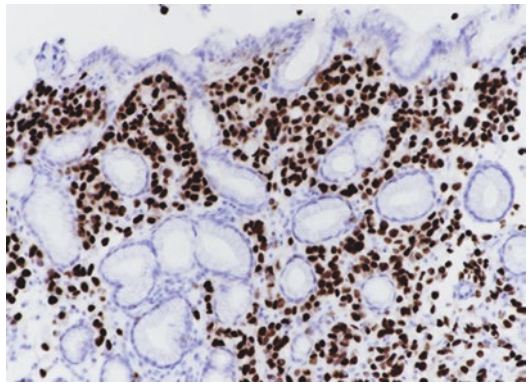

Fig. 10.2 Gastric mucosa infiltrated by metastatic breast carcinoma. Tumor cells with strong nuclear GATA-3 expression

els of GATA-3 in breast cancer predict a good prognostic outcome. GATA-3 as a marker for urothelial and other tumors is discussed in related chapters.

Diagnostic Pitfalls The expression of GATA-3 is not restricted to breast and urothelial tumors but is also found in a wide range of tissue and tumor types, which is to be considered in the interpretation of this marker [5]. Different expression intensity of GATA-3 is found in mesotheliomas, squamous cell carcinoma of different origin, pancreatic ductal adenocarcinoma, tumors of skin adnexa, and various types of benign and malignant salivary gland tumors, including salivary duct carcinoma, acinic cell carcinoma, adenoid cystic carcinoma, and epithelial-myoepithelial carcinoma [6, 7]. Minor cases of endometrium carcinoma are also reported to express GATA-3. Furthermore, the expression of GATA-3 is characteristic for T-lymphocytes and peripheral T-cell lymphomas. Noteworthy is the expression of GATA-3 in the epithelium of seminal vessels and reactive mesothelium, which can be a source of misinterpretation. Accordingly, GATA-3 is a multilineage marker that lacks specificity to breast and urothelial tumors, and the abovementioned notes must be considered in the interpretation of the GATA-3 stain.

10.3.2 Mammaglobin

Mammaglobin		
Expression pattern: Cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Breast carcinoma	Endometrioid adenocarcinoma; endocervical adenocarcinoma; salivary gland carcinoma (mammary analog secretory carcinoma, low-grade polymorphous adenocarcinoma, salivary duct carcinoma, adenoid cystic carcinoma and mucoepidermoid carcinoma); sweat gland carcinoma	Adult breast
Positive control: Normal breast tissue		

Diagnostic Approach Mammaglobin is a low molecular protein and a member of the secretoglobin-uteroglobin family, homologous to the human Clara cell protein expressed in adult breast tissue [8]. Mammaglobin is one of the most specific and sensitive markers for tumors of breast origin. The expression of mammaglobin is found in 80–90% of primary breast carcinoma and lymph node metastases [9, 10].

Diagnostic Pitfalls Similar to the other breast markers, the expression of mammaglobin is not restricted to breast tissue and breast tumors but can be found in a subset of other tumor types, including several types of salivary gland tumors, mainly mammary analog secretory carcinoma and low-grade polymorphous adenocarcinoma, in addition to endometrioid carcinoma, sweat gland carcinoma, and in a small subset of gastrointestinal cholangiocellular and pulmonary adenocarcinomas. Mesothelioma constantly lacks the expression of mammaglobin.

10.3.3 Gross Cystic Disease Fluid Protein 15

Gross cystic disease fluid protein 15 (GCDFP-15)		
Expression pattern: Cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Breast carcinoma – Primary Paget disease of the vulva	Salivary gland tumors, apocrine skin adnexal tumors, apocrine tumors, pulmonary adenocarcinoma, renal cell carcinoma, and ovarian and endometrial carcinomas	Apocrine-, lacrimal-, ceruminous-, Moll's-, and cutaneous eccrine glands; serous cells of submandibular, sublingual, and minor salivary glands; serous cells of nasal and bronchial glands
Positive control: Breast tissue/skin (apocrine cells)		

Diagnostic Approach **G**ross **c**ystic **d**isease **f**luid **p**rotein **15** (GCDFP-15, also known as BRST-2) is a prolactin-inducible glycoprotein initially isolated from the fluid of fibrocystic disease of the human breast. GCDFP-15 is expressed by apocrine cells or cells with apocrine metaplasia, regulated by the androgen receptor, and can be inhibited by anti-androgens [11]. In normal breast, ductal and lobular cells lack the expression of GCDFP-15. Antibody to GCDFP-15 reacts with apocrine cells of different origins and tumors arising from these cells. According to different reports, 30–90% of primary and metastatic breast carcinomas are positive for GCDFP-15. Triple-negative breast carcinoma is usually negative for GCDFP-15.

Diagnostic Pitfalls GCDFP-15 is one of the most specific markers for breast carcinoma; nevertheless, it is also expressed in other apocrine, eccrine, and serous glandular epithelium and carcinomas derived from these glands, including tumors of skin adnexa, which is to be considered in the differential diagnosis between primary skin tumors and metastases of breast carcinoma [12].

10.3.4 Tricho-Rhino-Phalangeal Syndrome 1 Protein (TRPS-1):

Tricho-rhino-phalangeal syndrome 1 protein (TRPS-1; also known as transcriptional repressor GATA binding 1) is a GATA-like zinc finger transcription factor encoded on 8q23.3 that binds to the GATA sequences and suppresses the transcriptional activity of GATA-regulated genes. TRPS-1 is an important regulator for the differentiation and proliferation of chondrocytes. Defects in this gene cause the autosomal dominant tricho-rhino-phalangeal syndrome (TRPS) type III characterized by craniofacial and skeletal abnormalities.

In normal tissue, nuclear TRPS-1 expression is found in squamous epithelium, ductal epithelial luminal cells of sweat glands and breast, gall bladder mucosa, glandular cells of the endometrium, prostatic glands, thyroid gland, and a subset of glial cells. In diagnostic immunohistochemistry, TRPS-1 is found to be more specific and sensitive than GATA-3 for breast carcinomas. Strong nuclear TRPS1 expression is found in more than 90% of receptor (ER/PR)-positive, HER2-positive, and triple-negative breast carcinomas, including different types of metaplastic breast carcinoma (Figs. 10.3 and 10.4). Contrary to GATA-3,

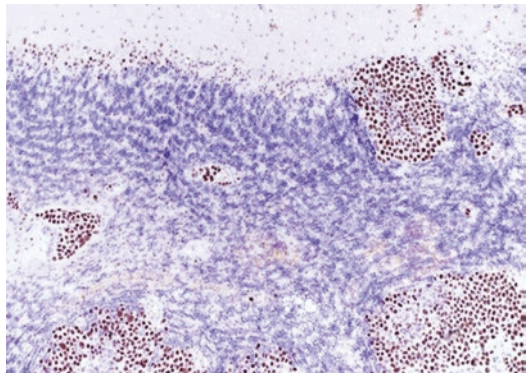

Fig. 10.3 Cerebellar metastasis of ductal breast carcinoma. Tumor cells with strong nuclear TRPS-1 expression

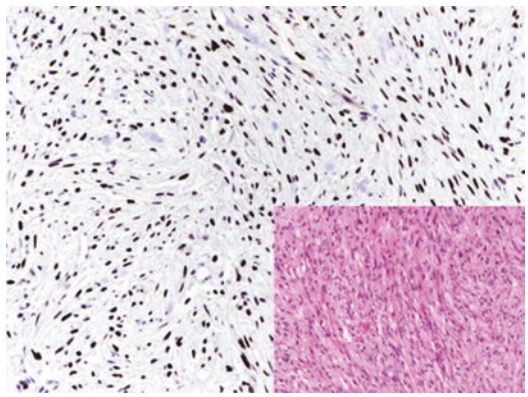

Fig. 10.4 Metaplastic breast carcinoma showing strong nuclear TRPS-1 expression in the tumor cells

TRPS-1 is usually negative or weakly positive in urothelial carcinoma. Furthermore, TRPS-1 is also a marker for mammary and extramammary Paget disease. Other adenocarcinoma types, including pulmonary, gastrointestinal, and pancreatic adenocarcinomas and ovarian and renal cell carcinomas, are also negative or very weakly positive for TRPS-1 [13–15].

Diagnostic Pitfalls TRPS-1 is reported to be negative in breast carcinomas with apocrine differentiation and in cutaneous apocrine carcinomas; both carcinoma types are usually positive for GATA-3 [16]. Similar to GATA-3, TRPS-1 is also expressed in squamous cell carcinomas of different origins, different salivary gland and sweat gland tumors and a subset of lymphocytes.

10.3.5 NY-Br-1

NY-BR-1 is a breast differentiation antigen expressed in normal breast epithelium and in up to 60% of breast carcinomas. The immunohistochemical reaction shows cytoplasmic and occasional nuclear stain patterns, and the expression intensity correlates with the differentiation of the tumor and the expression grade of estrogen receptors [17]. Sweat glands and about one-third of sweat gland tumors are also positive for NY-BR-1.

10.3.6 Estrogen Receptor

Estrogen receptor-α		
Expression pattern: Nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Breast carcinoma – Endometrial carcinoma	Ovarian serous, mucinous, and endometrioid carcinoma, transitional cell carcinoma, hepatocellular carcinoma, gastric adenocarcinoma, skin adnexal tumors, uterine leiomyoma and leiomyosarcoma, pituitary adenoma	Breast, endometrium, myometrium and endometrial stromal cells, fallopian tube mucosa, sweat glands, salivary glands, hepatocytes, pituitary gland
Positive control: Normal breast tissue		

Diagnostic Approach The estrogen receptor (ER) is a member of the steroid family of ligand-dependent transcription factors that include the estrogen, progesterone, and glucocorticoid receptors, in addition to the mineralocorticoid receptor. There are two types of nuclear estrogen receptors encoded by two different genes located on different chromosomes, the alpha type (ER-α) and beta type (ER-β), and each type includes different splice variants. Both types have different distributions in different organs and tissue types, whereas many tissue types show the expression of both receptor types [18].

The ER-α type, encoded by the ESR1 gene on chromosome 6q25.1, is mainly expressed in both epithelial and stromal cells of the breast, uterus, placenta, liver, hypothalamus, some types of pituitary adenoma, endothelium, and bone.

The ER-β type is encoded by the ESR2 gene on chromosome 14q23.2 and is mainly expressed in the prostate, testes, granulosa cells, spleen, thymus, skin, and endocrine glands, including the thyroid and parathyroid glands, adrenal glands, and pancreas.

The expression of estrogen receptors -α (ER-α) is a diagnostic marker for the majority of breast carcinomas in addition to tumors of uterine and ovarian origin; however, the expression of estrogen receptors may be found in other tumors such as hepatocellular carcinoma and tumors of the skin.

For the immunohistochemical stain, adequate and rapid tissue fixation with buffered neutral formalin is required for optimal stain results. For all steroid receptors, any stain pattern other than nuclear must be interpreted as negative. The expression of ER-α type is an important predictor for the response to the anti-hormone therapy (Fig. 10.5) [19].

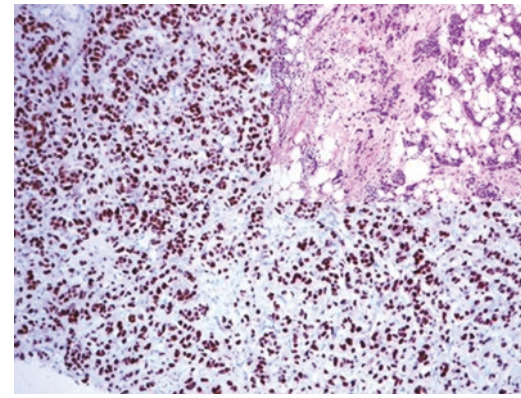

Fig. 10.5 Strong nuclear expression of estrogen receptors in breast carcinoma

During tumor progression, mutations can arise within the ESR1 gene causing resistance to aromatase inhibitors. These mutations usually appear in the ligand-binding domain and can be detected by molecular sequencing of the ESR1 gene.

Few scoring systems were suggested for semi-quantitative estimation of estrogen and progesterone receptors required to predict the response of different breast carcinoma types to specific endocrine therapy, including selective estrogen receptor modulators and aromatase inhibition. The modified scoring system introduced in 1987 by Remmele, the modified scoring system suggested in 1985 by McCarty, and the Allred scoring system proved to be the most practical and simplest systems. The three systems depend on the evaluation of the nuclear stain intensity and the percentage of positive tumor cells.

10.3.6.1 Remmele Scoring System

This simple scoring system has a 12-point scale (0–12) [19, 20]. To calculate the score, one of the numbers 0, 1, 2, or 3 is given according to the intensity of the nuclear stain and one of the numbers 0, 1, 2, 3, or 4 is given according to the percentage of positive tumor cells (see table). The score is calculated by multiplying the number reflecting the dominant stain intensity by the number reflecting the percentage of these positive tumor cells with a maximum score value of 12 (3x4) [21]. Tumors with a score of less than 3 usually respond poorly to anti-estrogen therapy.

10.3.6.2 Calculation of Remmele Score

Percentage of positive cells		Intensity of the stain	
0	No positive cells	0	No detectable stain
1	Positive cells less than 10%	1	Weak nuclear stain
2	Positive cells 10–50%	2	Moderate nuclear stain
3	Positive cells 51–80%	3	Strong nuclear stain
4	Positive cells of more than 80%		

10.3.6.3 McCarty Scoring System

This scoring system has a 300-point scale (0–300) [22]. The McCarty Histoscore is the total value of each percentage of positive cells (0–100) multiplied by the number reflecting the intensity of the immunohistochemical stain (0: no detectable

staining, 1: weak nuclear staining, 2: moderate nuclear staining, 3: strong nuclear staining) and calculated as the following:

- Percentage of tumor cells with strong positivity X 3 = **A.**
- Percentage of tumor cells with moderate positivity X 2 = **B.**
- Percentage of tumor cells with weak positivity X 1 = **C.**

The value of the Histoscore = **A** + **B** + **C.**

The clinical significance of this Histoscore is explained as the following:

50 or less: Negative (−).
51–100: Weakly positive (+).
101–200: Moderately positive (++).
201–300: Strongly positive (+++).

10.3.6.4 Allred Scoring System

The Allred scoring system has an 8-point scale (0–8). This scoring system is calculated by adding the number representing the proportion of positive cells 0, 1, 2, 3, 4, or 5 to the number reflecting the intensity of the nuclear stain 0, 1, 2, or 3 (see table). Tumors with a score of less than 3 usually respond poorly to anti-estrogen therapy.

10.3.6.5 Calculation of Allred Score

Percentage of positive cells		Intensity of the stain	
0	No positive cells	0	No detectable stain
1	Positive cells less than 1%	1	Weak nuclear stain
2	Positive cells 1–10%	2	Moderate nuclear stain
3	Positive cells 10–33%	3	Strong nuclear stain
4	Positive cells 33–66%		
5	Positive cells, more than 66%		

Diagnostic Pitfalls The expression of ER depends on the histological type and differentiation grade of the breast tumor. The expression of ER is not specific to breast and uterine tumors and also can be found in many others, such as hepatocellular carcinoma, gastric adenocarcinoma, and transitional cell carcinoma. Additional markers such as GATA-3, TRPS-1, mammaglobin, GCDFP15, and progesterone receptors, as well as the cytokeratin profile, help to confirm the diagnosis of primary breast carcinoma.

10.3.7 Progesterone Receptor

Progesterone receptor		
Expression pattern: Nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Breast carcinoma – Endometrial carcinoma	Skin adnexal tumors, meningioma, solid pseudopapillary tumor of the pancreas, stroma of mixed epithelial stromal tumor of the kidney, stromal tumors of the prostate, neuroendocrine tumors (mainly pancreatic)	Breast and endometrial cells, endometrial stromal cells
Positive control: Normal breast tissue		

Diagnostic Approach Progesterone is a steroid hormone involved in the differentiation of breast parenchyma and endometrium in addition to milk protein synthesis. The progesterone receptor (PgR) is a member of the steroid hormone receptor superfamily and estrogen-induced proteins that mediate the effect of the progesterone hormone expressed in different tissue types. PgR has three isoforms, A, B and C, all encoded by the same gene located on chromosome 11q22. PgR is a good marker for breast carcinomas and is more specific than estrogen receptors as it is expressed only in a limited number of tumors such as endometrial carcinoma, skin adnexal tumors, and meningiomas. The pro-

gesterone receptor status is one of the important prognostic factors for the management of breast, endometrial, and ovarian cancers [19]. A high expression level of both estrogen and progesterone hormone receptors is a positive prognostic factor for breast and endometrial cancers and predicts a good response to anti-estrogenic therapy.

Diagnostic Pitfalls Similar to the estrogen receptor, the expression of PgR depends on the grade of tumor differentiation. High-grade carcinomas are often negative for steroid receptors.

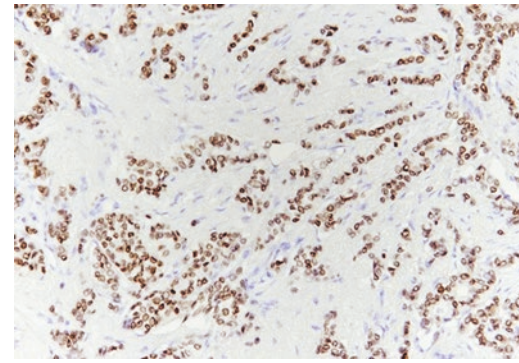

Fig. 10.6 Strong nuclear expression of androgen receptors in the neoplastic cells of invasive ductal carcinoma

10.3.8 Androgen Receptor

The androgen receptor (AR) is a nuclear receptor and a member of the steroid hormone receptor family, closely related to the progesterone receptor and activated by binding any of the androgenic hormones (testosterone and dihydrotestosterone). AR is variously expressed in different breast carcinoma types, and different expression levels are found in estrogen/progesterone/HER-2 positive and triple-negative breast carcinomas (luminal androgen receptor type) and is considered as one of the prognostic factors of breast carcinomas (Fig. 10.6) [23]. The strong expression of AR is one of the diagnostic characteristics of apocrine breast carcinoma and apocrine metaplasia. Metaplastic and mucinous carcinomas, in addition to basal-like and mesenchymal subtypes of triple-negative breast carcinomas, usually lack the expression of AR. AR is a potential therapeutic target in the luminal androgen receptor subtype of triple-negative breast carcinoma. The androgen receptor is also detailed in a later chapter (see Sect. 13.1).

10.3.9 Human Epidermal Growth Factor Receptor-2

HER-2 (c-erb-2)		
Expression pattern: Membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Breast carcinoma – Assessment of targeted HER-2 immunotherapy in different tumors	Gastrointestinal adenocarcinomas, carcinomas of the salivary glands, ovarian and endometrial carcinomas, a subset of pulmonary adenocarcinoma	Breast epithelium
Positive control: HER-2 positive tumors/brain tissue		

Diagnostic Approach Human epidermal growth factor receptor-2 (HER-2)—also known as p185, ERBB-2, or c-erbB-2 (**c**hicken **er**ythro**b**lastic viral oncogene homolog 2)—is one of the four members of the epidermal growth factor receptor family clustered as CD340, encoded on chromosome 17 (17q12). The HER-2 receptor consists of extracellular, transmembrane, and intracellular domains. In contrast to the other members of this family, HER-2 does not have a ligand-binding domain, and the activation of this receptor occurs by its dimerization. The HER-2 molecule is a part of the membrane of normal epithelial cells and $20x10^3$ to $50x10^3$ receptors are generally found on the surface of normal breast epithelial cells. During carcinogenesis,

the amplification of the HER-2 gene may occur, causing the overexpression of the HER-2 receptor, and up to $3x10^6$ receptors may be expressed on the membrane of these tumor cells. The HER-2 molecule is the therapeutic target in various tumors exhibiting the expression or overexpression of this receptor using specific antibodies or drug-conjugated antibodies. The overexpression of HER-2 is characteristic for various types of human carcinomas, mainly breast and gastric adenocarcinomas (Fig. 10.6), in addition to a subset of other carcinoma types such as ovarian carcinoma, non-small cell carcinoma of the lung, salivary gland carcinoma, and urinary bladder transitional cell carcinoma [24]. The amplification of the HER-2 gene can be detected by the FISH or CISH assay. A good alternative is semiquantitative detection using specific antibodies. Immunohistochemistry is an easy technique to estimate the corresponding overexpression of the HER-2 molecules on the membrane of the tumor cells. The immunohistochemical expression score is an important parameter for the immunotherapy of breast carcinomas and other HER-2-positive carcinomas. For the precise estimation of the HER-2 expression score, the following factors are to be considered:

- Only tissue with a cold ischemic time of less than 1 hour and optimal fixation (6–48 hours fixation)—preferably preoperative biopsies—is to be used for the HER-2 immunostaining.
- The interpretation of the immunostaining must begin with the evaluation of standardized control slides with the scores 0, 1+, and 3 + .
- Only membranous staining should be evaluated. Cytoplasmic or nuclear stains must be neglected. Staining caused by edge artifacts should also be ignored.
- Only invasive tumor components should be considered. The intraductal component may show a stronger signal than the invasive component, which can skew the evaluation.

The following table shows the criteria for the estimation of the HER-2 score in breast cancer. Note that the criteria for HER-2 score evaluation in other tumors vary and depend on the specimen type (see also HER-2 in gastric adenocarcinoma, Chap. 7).

10.3.9.1 Scoring of HER-2 Expression in Breast Cancer

IHC score	HER-2 overexpression	Staining result
0	Negative No gene amplification	No detectable staining (negative) or membrane staining in less than 10% of tumor cells
1+	Negative No gene amplification	A faint partial membrane staining in more than 10% of tumor cells
2+	Equivocal with uncertain gene amplification (see text below)	A weak to moderate staining of the entire membrane in more than 10% of tumor cells
3+	Positive High gene amplification	Strong staining of the entire membrane in more than 10% of tumor cells

According to the ASCO/CAP HER2 testing guidelines update 2018, breast carcinomas with the IHC HER-2 score 3+ are always classified as HER-2 positive and usually show a good response to the specific antibody therapy. Carcinomas with the scores IHC 1+ or 0 are to be classified as HER-2 negative with no evidence for gene amplification and are not sensitive to specific immunotherapy. However, score 1+ and non-amplified carcinomas with score 2+ can be classified as HER-2 low and maybe sensitive to drug-conjugated antibodies. Carcinomas with IHC HER-2 score 2+ need further confirmation by FISH/CISH assay or one of the molecular methods (real-time PCR or NGS). According to the ASCO/CAP HER-2 testing guidelines, the results of the FISH/CISH assays can be categorized into five groups: [25, 26] (Figs. 10.7 and 10.8)

- Group 1: HER-2/CEP17 ratio ≥ 2.0; average HER-2 gene copy number is ≥4.0/tumor cell. HER-2 status is categorized as HER-2 positive.
- Group 2: HER-2/CEP17 ratio ≥ 2.0; average HER-2 gene copy number < 4.0/tumor cell. HER-2 status is categorized as HER-2 negative unless IHC score 3 + .
- Group 3: HER-2/CEP17 ratio < 2.0; average HER-2 gene copy number ≥ 6.0 per tumor cell, probably due to trisomy or polysomy of chromosome 17, categorized as HER-2 negative unless IHC score 3 + .
- Group 4: HER-2/CEP17 ratio < 2.0; average HER-2 gene copy number > 4.0 and < 6.0/tumor cell, categorized as HER-2 negative unless IHC score 3 + .
- Group 5: HER-2/CEP17 ratio < 2.0; average HER-2 gene copy number is <4.0/tumor cell, categorized as HER-2 negative.

Diagnostic Pitfalls HER-2 is not a specific marker for breast tissue or breast carcinomas, and the overexpression of HER-2 is found only in up to 30% of breast carcinomas, mainly in high-grade carcinomas of no special type. Similar amplification may also be noted in other adenocarcinoma types of different origins.

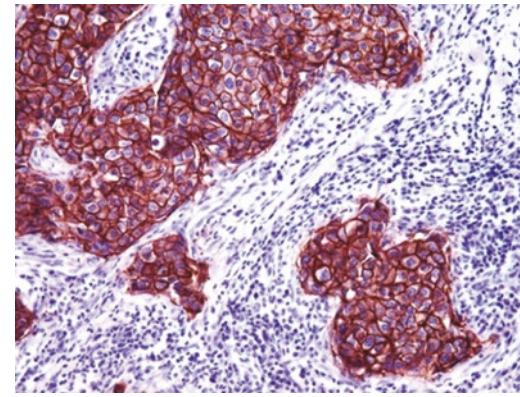

Fig. 10.7 Breast carcinoma with a strong membranous expression of HER-2 in all tumor cells (score 3+)

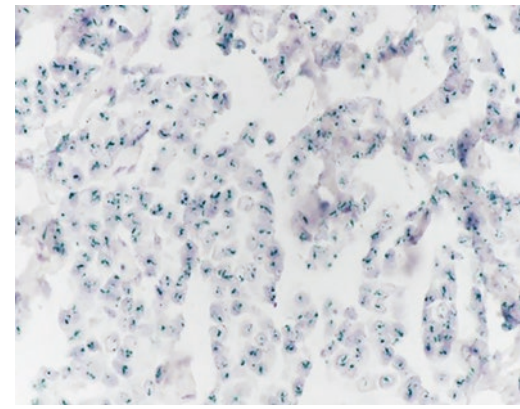

Fig. 10.8 CISH for HER-2 with high-level gene amplification with the formation of HER-2 gene clusters

10.3.10 Trophoblast Cell Surface Antigen 2

Trophoblast cell surface antigen 2 (Trop-2), also known as tumor-associated calcium signal transducer 2, is a type 1 transmembrane glycoprotein functioning as a calcium signal transducer. Low baseline Top-2 expression is found in different normal tissue types such as the breast, ovaries, pancreas, lungs, and kidney. During malignant transformation, the expression of Trop-2 is upregulated, and overexpression of Trop-2 is noticed in different carcinoma types, including gastrointestinal, pulmonary, genitourinary, and breast carcinomas. In most tumors, the overexpression of Trop-2 correlates with aggressive behavior and poor prognosis.

As a cell surface protein, Trop-2 is an interesting target for specific humanized therapeutic antibodies and specific inhibitors used to treat different carcinoma types exhibiting Trop-2 overexpression, such as triple-negative breast carcinoma (see also Sect. 14.3).

10.3.11 E-Cadherin

E-cadherin is a transmembrane glycoprotein and a member of the cadherin superfamily functioning as an adhesion molecule listed in detail in a previous section (see Sect. 2.4).

In routine histopathology, E-cadherin is a helpful marker to discriminate between ductal and lobular breast neoplasms as lobular breast carcinomas, including lobular carcinoma in situ,

lack the expression of E-cadherin due to mutations within the gene encoding E-cadherin (Fig. 10.9). These mutations cause the synthesis of anomalous E-cadherin molecule without cohesiveness properties, which also cannot be detected by the standard antibodies or might show atypical intracytoplasmic or perinuclear expression pattern. The absence of normal E-cadherin in the cells of lobular neoplasms leads to the intracytoplasmic accumulation of δ1- Catenin (also known as p120), making it a further interesting marker for lobular carcinomas of the breast with intense cytoplasmic stain. Myoepithelial cells surrounding non-invasive carcinomas are generally positive for E-cadherin [27] (Fig. 10.10). E-cadherin is also a prognostic marker for various carcinoma types such as breast, poorly cohesive gastric adenocarcinoma, and transitional carcinoma, as the loss of E-cadherin expression is found to be associated with aggressive behavior.

Diagnostic Pitfalls The correlation with the tumor morphology is essential as the loss of E-cadherin expression may be found in a subset of poorly differentiated carcinomas. It is also important to consider that up to 15% of invasive lobular breast carcinomas may show E-cadherin expression in different intensities.

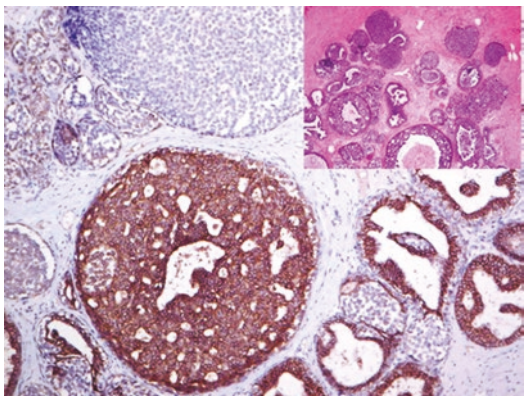

Fig. 10.9 E-cadherin highlighting the neoplastic cells of ductal carcinoma in situ (DCIS), whereas the cells of lobular carcinoma in situ (LCIS) lack the E-cadherin expression

10.3.12 Smooth Muscle Myosin Heavy Chain

Smooth muscle myosin heavy chain (SMMHC) is a structural contractile protein listed with the markers of smooth muscle tumors (Sect. 24.2). In breast pathology, SMMHC is a helpful marker that selectively highlights the myoepithelial cells usually preserved as a continuous layer surrounding benign breast lesions (Fig. 10.11). This assay is important to discriminate between invasive breast carcinomas (tubu-

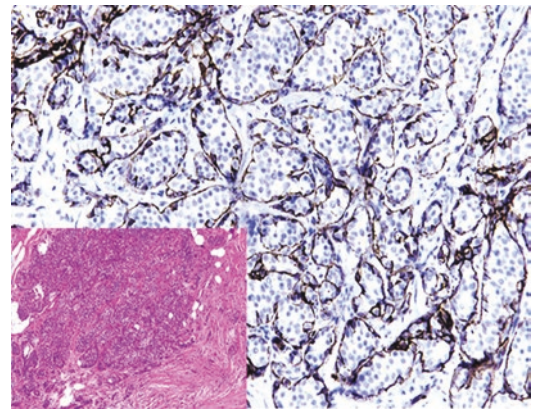

Fig. 10.10 Lobular carcinoma in situ surrounded by E-cadherin positive myoepithelial cells. Luminal neoplastic cells are negative for E-cadherin

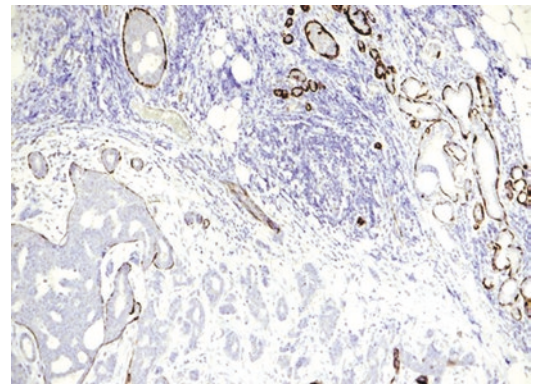

Fig. 10.11 Myosin stain of breast tissue. Benign areas and non-invasive (DCIS) structures with retained myoepithelial cells in the periphery and invasive portions lacking the myoepithelial cell layer

lar, papillary, and cribriform carcinoma) lacking the myoepithelial cell layer and other non-invasive, benign, or reactive lesions such as ductal carcinoma in situ (DCIS), radial scar, benign hyperplasia, and adenosis besides papilloma and nipple adenoma typically with intact myoepithelial cell component.

10.3.13 Assessment of Triple-Negative Breast Carcinoma

Triple-negative breast carcinoma is a heterogeneous group of breast tumors that lacks the expression of HER-2 and both estrogen and progesterone receptors and accounts for 10–15% of all breast tumors. This tumor group includes low-grade and high-grade carcinoma identities. The high-grade group contains several subtypes, including carcinoma with basal-like phenotype, carcinoma with apocrine differentiation, and metaplastic carcinoma. The low-grade carcinoma identities include secretory carcinoma, breast carcinomas of salivary gland type, and tall cell carcinoma with reversed polarity. The triple-negative immunophenotype is suspicious of BRCA1 mutation as ~60% of breast carcinomas associated with BRCA1 are triple-negative.

Several molecular subtypes of triple-negative breast carcinoma of no special type are described and can be assessed using special immunohistochemical markers. As reported in several studies, these subtypes have different biological and clinical behavior, different responses to therapy protocols, and include the following identities [28–31]:

- Basal-like immune suppressed type: carcinoma with high genetic instability.
- Immunomodulatory type (basal-like immune activated): carcinoma with high genetic instability. Tumor tissue enriched by tumor-infiltrating inflammatory cells, including CD8 positive T-lymphocytes and CD20 B- lymphocytes.
- Mesenchymal type: carcinoma with intermediate genetic instability.
- Luminal androgen receptor type: hormonally regulated carcinoma with strong expression of the androgen receptors and low genetic instability.

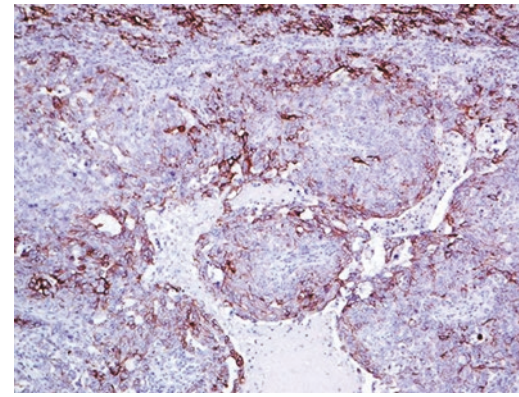

Fig. 10.12 Strong cytoplasmic CK 5/14 expression in triple-negative basal type breast carcinoma

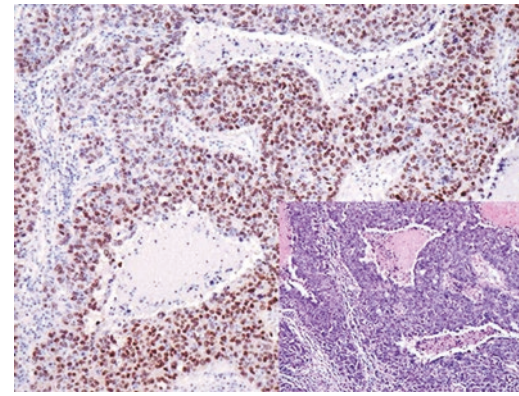

Fig. 10.13 Nuclear Sox-10 expression in neoplastic cells of triple negative basal-type breast carcinoma

Triple-negative breast carcinoma with basal-like phenotype is the most common triple-negative carcinoma type characterized by the expression of high molecular weight Cytokeratins (CK5/6/14/17), besides EGFR, Sox-10, and FOXC1 and usually associated with p53 accumulation (Figs. 10.12 and 10.13). It is also important to consider that high molecular weight Cytokeratins also stain metaplastic carcinoma and Sox-10 stains secretory carcinoma.

TRPS1 and GATA-3 are expressed in the majority of triple-negative breast carcinomas, while p40 and p63 have only a little diagnostic value as they are frequently negative. p16 and FOXC1 are strongly expressed in the basal-like immune-suppressed subtype.

Immunoprofile of breast tumors				
Tumor types	+ in > 90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in < 10% (−)
Ductal hyperplasia (usual ductal hyperplasia; UDH):	UDH is composed of heterogeneous cell populations: 1. Glandular epithelial cells: positive for CK7, CK8, CK18, and CK19 2. Intermediate myoepithelial cells positive for CK5/6, CK14, CK8, CK18, CK19, and Actin 3. Intact continuous layer of myoepithelial cells positive for CK5/6, CK14, p63, Actin and Myosin Intact basement membrane positive for Laminin			
Atypical ductal hyperplasia (ADH):	1. Clonal proliferation of luminal glandular epithelial cells: CK7, CK8, CK18, and CK19 2. No luminal or only rare residual CK5/6/14, p63 positive intermediate myoepithelial cells 3. Intact continuous layer of myoepithelial cells positive for CK5/6, CK14, p63, Actin and Myosin 4. Intact basement membrane positive for Laminin Luminal glandular cells are positive for Estrogen (ER) and Progesterone (PgR) receptors and negative for HER-2 and p53			
Ductal carcinoma in situ[a] (DCIS) low grade:	CK7, CK8, CK18, CK19, ER Preserved basal myoepithelial cell layer positive for myoepithelial markers (p63, CK5/6/14, Actin, Myosin)	PgR, bcl-2	HER-2, p53, Cyclin D1	
Ductal carcinoma in situ[a] (DCIS) high grade:	CK7, CK8, CK18, CK19, **E-Cadherin** Preserved basal myoepithelial cell layer positive for myoepithelial markers (p63, CK5/6/14, Actin, Myosin)	Cyclin D1, HER-2, p53	ER, PgR, bcl-2	
Lobular carcinoma in situ[a] (LCIS):	CK7, CK8, CK18, CK19, GATA-3 Preserved basal myoepithelial cell layer positive for myoepithelial markers (p63, CK5/6/14, Actin, Myosin)	ER expression in 80–95%		CK20, p53, **E-cadherin**[b], HER-2 Cyclin D1
Invasive carcinoma of no special type (NST) A. Invasive ductal carcinoma NOS.	CK7, CK8, CK18, CK19, EMA[c], CD44, **GATA-3**, **E-Cadherin**, β-Catenin, p120 Catenin (m)[d]	Maspin, human milk fat globule, EGFR ER expression in 70–80% PgR in 70–80%	GCDFP15, Bcl-2, CK10/13 HER-2 overexpression in 15–20%	CK14, CK17, CK20
Invasive carcinoma of no special type B. Oncocytic carcinoma.	CK8, CK18, EMA	CK7, ER expression in 70–80% PgR in 60–70%	HER-2 overexpression in ~25%	HER2, GCDFP-15
Invasive carcinoma of no special type C. Lipid-rich carcinoma.	Adipophilin	HER-2 overexpression in 75–100%		CK5/6/14, ER, PgR
Invasive carcinoma of no special type D. Glycogen-rich carcinoma.			ER expression in 35–50%	PgR

Invasive carcinoma of no special type E. Sebaceous carcinoma.	ER, PgR and HER-2 expression in ~60%			
Invasive carcinoma of no special type F. Basal-like phenotype.	CK5/6/14, p40, p63, **Sox-10**, EGFR	Vimentin, CK17		ER, PgR, HER-2
Invasive carcinoma of no special type G. Medullary carcinoma and carcinoma with medullary features.	CK 8, CK 18	p53, EGFR	Vimentin, S100, CK5/6, CK14	HER-2, CK7, CK19, CK20, GCDFP15 ER and PgR[e] expression in 0–10%
Invasive lobular carcinoma:	**CK7**, CK8, CK18, CK19, **GATA-3**, **p120 Catenin**[d]	GCPF15, CEA, Cyclin D1, Maspin, ER expression in 80–95% PgR in 80–90% AR in 80%	NKX3.1, EGFR	**E-Cadherin**, HER-2 (<5%), CK5/6, CK14, CK20
Tubular carcinoma:	**CK7**, CK18, CK19, **GATA-3** Absence of basal myoepithelial cell layer positive for myoepithelial markers (p63, CK5/6/14, Actin, Myosin)	ER and PgR expression in 90–100%		HER-2, CK5/6, CK20
Cribriform carcinoma:	**CK7**, CK8, CK18, CK19, **GATA-3**	Human milk fat globule ER expression in 90–100%, PgR expression in ~70%	CK10/13	HER-2, CK14, CK20
Mucinous carcinoma:	CK7, CK18, CK19, MUC-2, CEA	WT-1, androgen receptors, NSE, chromogranin, Synaptophysin, ER expression in ~90%, PgR in 70–80%	EGFR	HER-2, CK20, CDX-2
Mucinous cystadenocarcinoma:	CK7, CK18		CK5/6/14 ER and PgR expression in <20%	HER-2, CK20, CDX-2
Invasive micropapillary carcinoma:	CK7, CK18, CK19, **EMA**[e] ER and PgR expression in ~90–100%	LEF-1		
Invasive papillary carcinoma:	CK7, CK18, CK19, CEA			CK5/6, CK14
Solid papillary carcinoma:	CK7, CK18 ER and PgR expression in ~90–100%	Chromogranin, Synaptophysin		HER-2, CK5/6/14
Carcinoma with apocrine differentiation:	CK8, CK18, CK19, **Androgen receptors**	**GCDFP15**, CEA, ER-β	HER-2 (amplification in 30–60%)	ER-α, PgR, S100
Metaplastic carcinoma:	Vimentin, Pan-CK	CK7, CD44	EMA, Actin, S100, GATA-3	ER, PgR

Breast tumors of salivary gland type: – Adenoid cystic carcinoma – Acinic cell carcinoma – Mucoepidermoid carcinoma – Polymorphous carcinoma	See salivary gland tumors (Sect. 6.2) Usually triple negative carcinomas			
Secretory carcinoma:	CK8, CK18, CK19, EMA, Lactalbumin, **S100**, **Sox-10**, **NTRK**[f]	CK5/6/14, S100, GATA-3, CEA, EGFR, Vimentin	CD117	ER and PgR expression in <10% HER-2
Tall cell carcinoma with reversed polarity:	CK5/6/14, CK7, **IDH-2$_{R172S}$**	GATA-3, GCDFP-15	AR	ER and PgR expression in <10%
Neuroendocrine tumors of the breast: – Carcinoid (NET G1 and G2) – Small cell carcinoma (NET G3/NEC) – Large cell neuroendocrine carcinoma (NEC)	See neuroendocrine tumors and neuroendocrine carcinomas (Chap. 14) Usually triple negative carcinomas			
Phyllodes tumor:	*Stromal cells*: Vimentin *Epithelial cells*: CK 5/6, CK14, CK8/18, Pan-CK, EMA Proliferation index (Ki-67) in stromal cells: In benign type usually <20% In malignant type usually >20%	CD34, bcl-2, CD117, p53 CEA	CD34, actin, Desmin, CD10, CD117	S100, pan-CK, EMA
Myofibroblastoma of the breast:	Desmin, CD34, CD99, bcl2, Vimentin	CD10, Actin, Androgen receptors	PgR, ER	Pan-CK, S100
Tumors of the nipple				
Paget's disease of the nipple:	**CK7**, [g] CK8, CK18, EMA (MUC-1), CD63 (NK1-C3)	CEA, GCDFP15, **HER-2**[g]	ER, PgR, AR	CK5/6, CK20, MUC-2
Syringomatous tumor:	CK5/6/14, CK8, CK18, p40, p63		ER	PgR, HER-2

[a] No luminal or only residual of CK5/6/14 positive intermediate myoepithelial cells. An intact layer of basal myoepithelial cells positive for CK5/6, CK14, p63, Actin and myosin, h-Caldesmon, or Calponin

[b] E-Cadherin is positive in normal non-neoplastic breast lobular cells

[c] In invasive micropapillary carcinoma, the reverse cell polarity with inverted EMA expression on the basal surface is characteristic; other breast tumors show the EMA expression on the apical surface of tumor cells (see Fig.10.14)

[d] P120 Catenin shows a membranous stain in invasive ductal carcinoma but a characteristic cytoplasmic stain in lobular carcinoma

[e] ER and PgR are usually negative in typical medullary carcinoma

[f] Secretory carcinoma is usually associated with the t(12;15)(p13;q25) translocation generating the ETV6-NTRK gene fusion

[g] See Figs. 10.15 and 10.16

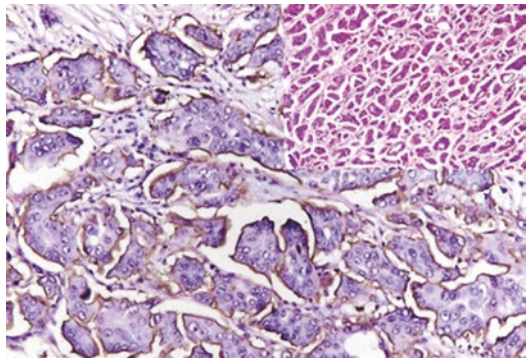

Fig. 10.14 Characteristic inverted (basal) EMA expression in invasive micropapillary carcinoma

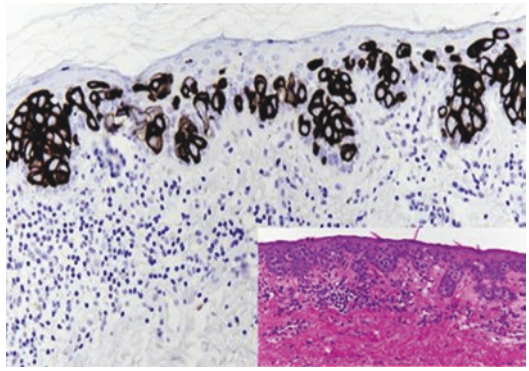

Fig. 10.15 Paget's disease of the nipple. Intraepidermal tumor cells with strong cytoplasmic CK7 expression

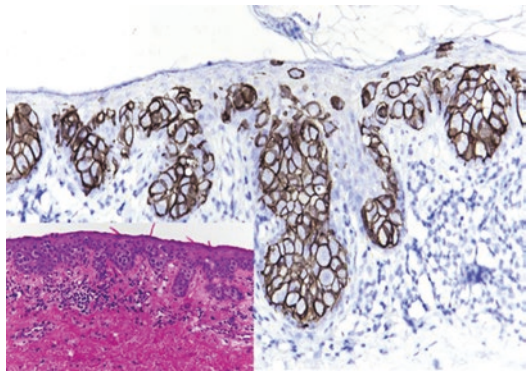

Fig. 10.16 Paget's disease of the nipple. Intraepidermal tumor cells with strong membranous HER-2 expression

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