Molecular Pathology of Breast Cancer

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Introduction

- Breast cancer is the most common cancer affecting women, with an estimated 250,000 new cases in 2011 in the US alone and 1.5 million worldwide. It is one of the first major diseases where basic laboratory research has had a large impact on the routine clinical management of patients, ranging from detection, to diagnosis, to therapy. Molecular approaches to pathology, in particular, have had an enormous influence, especially in the areas of diagnosis and therapeutic decision-making. The topic of molecular pathology in breast cancer is very large and evolving far too rapidly to cover completely in a chapter of this nature. This chapter will primarily focus on reviewing aspects that are already in routine clinical use, some of the more promising applications on the near horizon, and scientific questions that are currently at the forefront of translational research. From an etiological point of view, the molecular pathology of breast cancer is the result of molecular abnormalities occurring in important normal processes, including the gross, microscopic, and molecular anatomy of the breast, breast development, and adult physiology—which is where we begin

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Normal Characteristics of the Female Human Breast

Gross, Microscopic, and Molecular Anatomy

- Grossly, the size of the adult female breast varies enormously. On average, it is about 10–12 cm in diameter, 5–8 cm in thickness, and weighs about 700 g. Weight may almost double during pregnancy and lactation. Pathologists typically divide the breast into four quadrants (Q): upper outer (UOQ), upper inner (UIQ), lower outer (LOQ), and lower inner (LIQ). Other important regions are the areola/nipple complex and the lymph nodes in axillary tail extending from the UOQ. Lymphatic (and vascular) drainage is important as the main pathway for breast cancer cells to metastasize. Most regions of the breast, especially the UOQ and LOQ, drain to the axillary nodes, although the LIQ and UIQ also drain to a chain of internal mammary nodes beneath the sternum and extending upwards
- Internally, the breast is composed of 15–20 segments or lobes, somewhat analogous to segments of an orange, but less well defined. Each lobe contains thousands of lobules, which are small grape-like clusters of glands lined by epithelial cells specialized to produce milk. Small ducts that join to form larger ducts that eventually exit through the nipple, transmitting milk to nourish our young, interconnect the lobules. All known precursors of breast cancer, also referred to as premalignant lesions, develop and progress from abnormal cells within the ductal system, primarily in the lobules and smallest ducts connected to them, referred to as the terminal duct lobular unit (TDLU)
- The entire normal ductal and lobular system is delineated from the mesenchymal stroma ("connective tissue") by a continuous basement membrane (BM) which is an important barrier which must be breached for cancer cells to invade and metastasize
- The lumens of the ducts and lobules are generally lined by two distinct layers of cells; an outer layer directly on top of the BM referred to a myoepithelial cells (MECs), and an inner layer directly on top of the MECs referred to a luminal epithelial cells (LECs)—although LECs also have many subtle points of attachment with the BM interspersed with the MECs
- Nearly, all LECs typically express large amounts of keratin proteins, particularly CK8, CK18, and CK19. MECs express abundant CK5 and CK6, but are generally negative for keratins found in LECs, and they do not express ER or PR. MECs also typically express several other molecules distinct from LECs, including smooth muscle actin (SMA), calponin, S100, p63, CD10, and stratifin (SFN), which appear to be important in certain specialized normal functions such as contraction of duct lumens to expel milk, and to maintain normal cell polarity within ducts, which can actively suppress the invasion of cancer cells
- These keratins play an important role in a new molecular classification of breast cancers—the so-called intrinsic molecular subtypes, which is discussed in more detail later. Briefly, the most common subtype is referred to as "luminal" breast cancers, primarily because they have many similarities at the gene expression level with normal LECs, including these keratins. Another important subtype, referred to as "basal" breast cancers, expresses keratins normally associated with MECs, which historically have been referred to as "basal" cells because of their location in ducts and lobules. There is a common misconception that luminal and basal breast cancers evolve from genetically altered LECs and MECs, respectively, partly because of molecular similarities including keratins—which is probably not true, although the "stem" cell origin of all breast cancers is far from clear and a topic of much debate and research
- A proportion of LECs (20–30%) also express nuclear estrogen receptors (ER) and progesterone receptors (PR). ER and PR are important mediators of growth and differentiation stimulated by the hormones estrogen and progesterone. The majority of cancer cells also

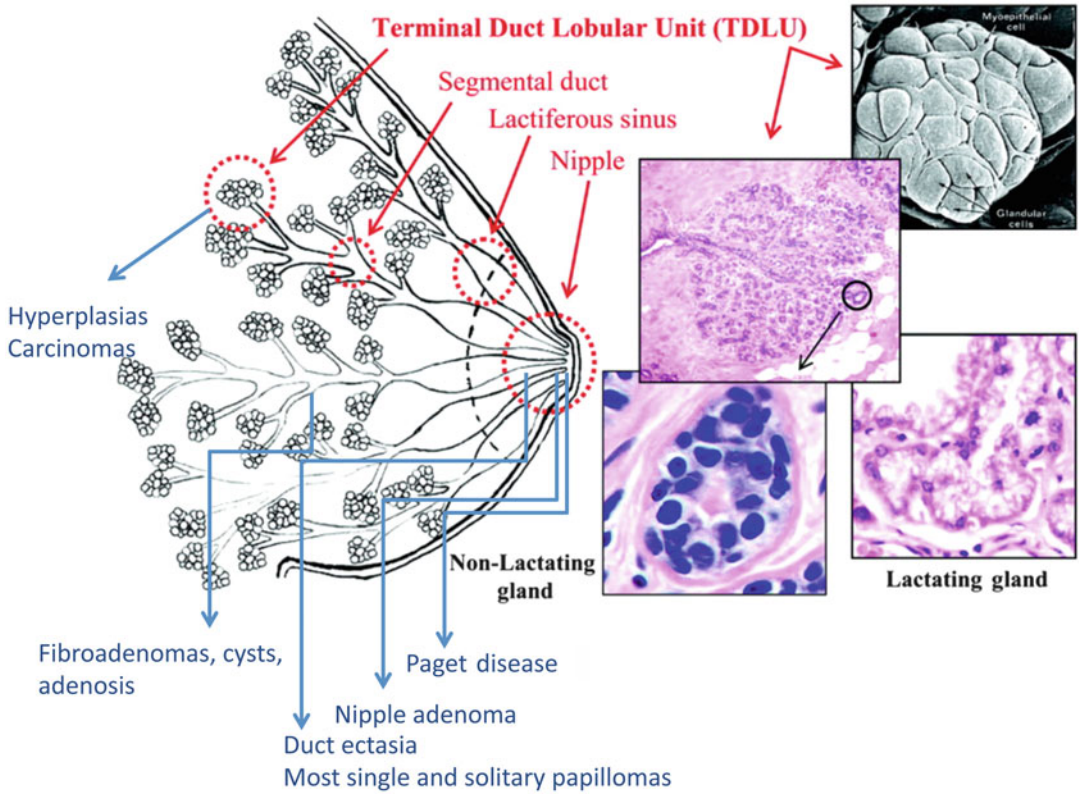


Fig. 6.1 Anatomy of the adult mature human breast. Correlation between compartments and different distinct pathologic processes arising in the breast

express these receptors, which may promote tumor growth

- Recent studies have shown that histologically normal appearing breast epithelial cells are not always normal at the molecular level, and some of these morphologically silent biological abnormalities may predispose the cells to premalignant or malignant transformation. For example, chromosomal gains and losses have been observed in normal breast epithelium. Although the overall frequency of imbalances is quite low, it is significantly higher in normal cells adjacent to cancer cells than normal cells at a distance. Some of these genetic defects may be shared with the adjacent cancer, although the majority are not and appear to be random. Other studies have shown that breast tissue, especially in women at high risk for breast cancer, may contain patches of histologically normal appearing cells in which activity of the p16 tumor suppressor gene is suppressed. Compared to adjacent cells with normal p16 function, these cells show increased proliferation and elevated expression of cyclooxygenase 2 (COX2), and the latter appears to be associated with the development of many types of cancers. There are likely to be many other acquired and inherited molecular abnormalities in otherwise normal appearing cells (Figs. 6.1 and 6.2)

Breast Development

- The molecular mechanisms responsible for human breast development are poorly understood because it is extremely difficult to study directly. Most of what we know is inferred

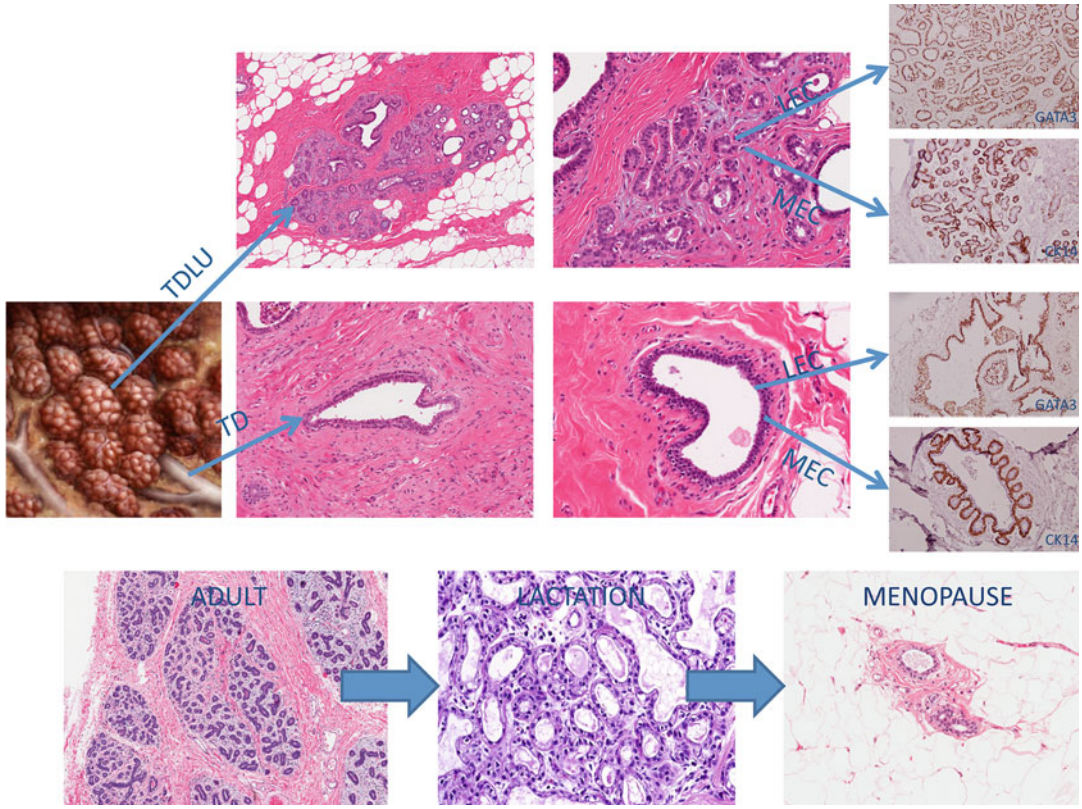


Fig. 6.2 Breast histology. Differences between luminal epithelial cells (LEC) and myoepithelial cell layer (MEC) compartments. GATA 3 is a representative marker of LEC in both TDLU and TD. CK14 is a distinctive marker of MEC. *Lower*: Histological changes associated with lactation and menopause. During lactation, the acini are closely packed, with reduced amount of stroma; secretory material in the lumens is seen. With menopause, there is a marked reduction of acini and ducts, with replacement by fat

from animal studies, particularly involving genetically engineered mice, where the effect of altering specific genes on breast development can be directly observed. However, there are probably many important parallels in breast development among all mammals, and studies using mice and other models almost certainly reveal molecular mechanisms shared with humans. Many normal developmental mechanisms play a central role in the development and progression of breast cancers. For example, cells in the earliest potential precursors of breast cancer, referred to as hyperplasias, demonstrate suppression of molecular pathways involved in adult differentiation, and reactivation of embryonic pathways, which is also true of later stages such as the progression of ductal carcinoma in situ (DCIS) to invasive breast cancer (IBC)

- Mammary glands are derived from ectodermal buds or ingrowths along mammary lines in the embryo. Between 14 and 18 weeks of gestation, distinct mesenchymal and ductal compartments start to develop. By 28 weeks, there are two clearly defined cell compartments (LECs and MECs). The ductal and lobular system continues to develop and mature throughout the second half of gestation, as well as the areola and nipple. Many genes are known to play critical roles in regulating development. For example, BCL2, which suppresses apoptosis, increases dramatically beginning at about 18 weeks, and plays a important role in duct formation by inducing

Table 6.1 Genes involved in breast development

Gene	Disease	Pathway	Clinical features
TBX3	Ulnar mammary syndrome	Linked to FGF pathway	Abnormalities in limbs and apocrine glands TBX3 overexpression linked to breast carcinomas
PTHR1	Blomstrand chondrodysplasia	Mutation in receptor or parathormone (PTH) mediates cross-talk between epithelium and mesenchyme in early mammary bud	Dwarfism PTHrP is commonly secreted in breast cancers
Ectodysplasin	Hypohidrotic ectodermal dysplasias	Development of ectodermal appendages	X-linked ectodermal dysplasia receptor (which binds ectodysplasin) promoter methylation is linked to breast cancer
Ska (*neuroregulin 3*)	None known	Affects patterning of mammary glands, along the body axes. NRG-3 is a ligand to EGFR family	Upregulated in breast cancer, particularly those with HER2 overexpression
WNT		LEF1, the transcriptional mediator of WNT signaling at placode stage	LEF1−/− embryos placodes 2 and 3 do not form; the other placodes develop into small buds and degenerate. Corresponding ducts and nipples are missing in newborn

Clinical consequences of mutations involving those genes and associated clinical syndromes

cells in the center of solid cords of primitive epithelial cells to die, forming patent lumens. Ductal budding and branching depends on prolactin which sensitizes cells to the growth stimulating effects of insulin. Aldosterone promotes differentiation of buds into ducts and lobules, forming primitive TDLUs. ER is expressed in LECs by third trimester and PR, 2–3 months after birth. Genetic alterations of these regulatory molecules can play important roles in the development and progression of breast cancer, in general, by promoting "embryonic" growth in an inappropriate setting. Other important genes are discussed later in the context of what happens to breast development when they are altered in transgenic and knockout mice (Tables 6.1 and 6.2)

- There are no structural or known molecular differences between male and female breasts during the postnatal period. At birth, nipple ducts finally open onto the surface. Closely after birth, prolactin, estrogen, and progesterone decrease dramatically, resulting in involution of newborn breast tissue. During this time, apocrine and cystic changes become prominent, which are also common in postmenopausal breasts. Between 2 years of age and puberty, the breasts are very small, and the main constituents are scattered small ducts embedded within a dense collagenous stroma. Pubertal changes are characterized by greatly increased growth of stroma, MECs, and LECs, which are prominently caused by increased levels of estrogen, although full differentiation requires other hormones and growth factors as well, including insulin, cortisol, thyroxin, prolactin, and growth hormone. ER is necessary for duct elongation, and ER knockout mice only develop rudimentary ducts without terminal end buds or alveolar buds. Interestingly, these glands are highly resistant to cancer development. PR is necessary for duct elongation and alveolar development, which are lacking in PR knockout mice. After menarche, prominent cyclical developmental changes occur with the menstrual cycle. Early on,

Table 6.2 Animal transgenic models in breast carcinogenesis

Gene (KO or overexpression)	Pathway	Clinical features
BRCA1 KO	BRCA1 and p53	Increased mammary tumor development in BRCA1 KO that was p53 heterozygous (p53+/−) suggested that BRCA1 loss may induce tumor development due to genetic instability causing LOH LOH in p53 was seen in majority of BRCA1 KO mice
Erα OE	ER	Mammary carcinomas with similarities to human breast cancer and ER+ phenotype
Aromatase OE	Aromatase	Male mice developed gynecomastia, and homozygous mice were infertile and developed Leydig testicular cancers Females developed ductal hyperplasias and dysplasia. However, no mammary tumors were seen Mice exhibited increased ERα and ERβ levels, as well as PR, cyclin D1, and cyclin E levels (cyclin D1 overexpression correlates with ER+ phenotype in human cancers) DMBA treated mice with AO developed mammary tumors, whereas WT only showed hyperplasia. Letrozole effectively inhibited dysplastic growth in MMTV-aromatase mice
TGFα/HER2	TGFα and HER2	Double transgenic mice developed significantly less breast tumors than parental lines. Double transgenic mice with HER2 aromatase overexpression show less hyperplasias
ERα KO	ER	Mammary glands resembled prepubertal wild-type mice. ERα KO mammary epithelium underwent ductal morphogenesis when transplanted to wild-type mice. Transgenic mice with MMTV-aromatase/ERα KO did not develop hyperplastic growth and exhibited morphology similar to ERα KO mice. ERα mediated growth of the mammary duct network is a prerequisite for aromatase induced changes within the transgenic mammary gland
WNT	WNT	Mice developed ductal hyperplasias early in life and mammary adenocarcinomas in most animals by 1 year of age. MMTV-wnt/ERα KO−/− exhibited stunted growth similar to parental ERα KO mice
PELP-1	Coactivator of ER, PR, AR. Mediates G1-S transition. Aromatase pathway	MMTV-PELP1 developed mammary tumors in over 40% of cases. Tumors show ER and aromatase expression. Human breast cancers commonly show PELP1 overexpression and are associated with poor response to tamoxifen
AIB1 KO/OE	Binds to steroid receptors and transcription factors	AIB-1 levels have been correlated with poor prognosis in breast cancer. Coinduction of AIB1 and HER2 was associated with decreased DFS and tamoxifen resistance. AIB1 KO resulted in decreased oncogenesis with decreased HRAS, HER2, and IGF1 expression. MMTV-AIB1 resulted in tumor development in 48% of mice. The carcinogenic potential was abrogated in double transgenic mice with MMTV-AIB1 ERα KO (ER is important in the AIB1 pathway). Induction of IGF1 signaling in the mammary gland is typical of the AIB1 transgenic model. Treatment with the mTOR inhibitor RAD001 resulted in block of hyperplasia and atypia in the AIB1 transgenic model
CSF1	CSF1	The macrophage colony-stimulating factor, CSF1, is commonly elevated in breast cancer. CSF1 op/op is deficient in lactation and develops osteopetrosis. Cross-linked species of MMTV-PYMT CSF op/op showed less progression of disease and lung metastases compared to the parental strain of MMTV-PYMT

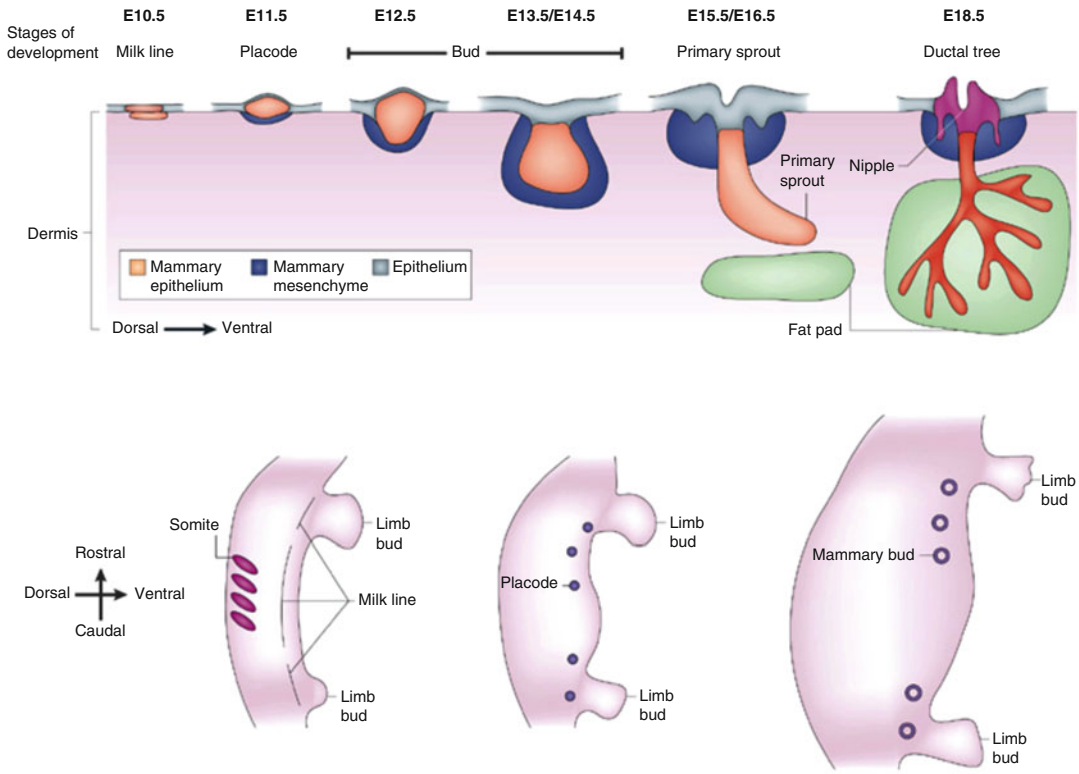
TDLUs develop more alveoli with each successive cycle. From menarche on, the mammary gland is fully anatomically and functionally developed to support pregnancy and lactation

- During pregnancy, proliferation of essentially all types of cells, especially LECs, dramatically increases, mediated by increasing levels of estrogen, progesterone, ER, and PR. After delivery circulating ER and PR decrease to low levels, in preparation for lactation. Once lactation begins, cell proliferation ceases as the cells terminally differentiate to produce milk. When lactation ceases, secretory LECs undergo apoptosis, alveoli collapse, and the mammary gland involutes back to the nonpregnant condition, although the ductal system postpregnancy retains a somewhat more complex ductal framework than prior to pregnancy. In the adult female breast, there is a relatively large reserve of normal stem cells which support the dynamic changes in growth and differentiation associated with menstrual cycling, pregnancy, and lactation. Presumably, various genetic alterations of normal stem cells may give rise to precancerous or cancer-stem cells, which eventually grow uncontrollably. However, there are probably other sources of cancer-stem cells, including dedifferentiation of mature LECs due to specific mutations
- After menopause, both lobules and ducts are decreased in number. Intralobular stroma is replaced by collagen and the breast stroma undergoes replacement by fat (Fig. 6.3)

Molecular Biomarkers in Routine Clinical Practice

Estrogen Receptor and Progesterone Receptor: Molecular and Clinical Aspects

- The measurement of ER and PR has become a standard of practice in the evaluation of patients with primary breast cancer. The measurements can be performed accurately on formalin-fixed paraffin-embedded (FFPE) tissue by using immunohistochemistry (IHC) and the results have good correlation with those of biochemical testing
- The ER is the paradigm tumor marker for management of patients with cancer. It dates back to at least 1896 when G Batson reported regression of advanced breast cancer in women who underwent oophorectomy
- ER controls essential developmental and physiological processes. It interacts with the receptor as estradiol, regulates growth and differentiation, and helps maintain homeostasis. Studies have shown that dysregulation of ER and PR during development are important in carcinogenesis
- The effects and actions of estradiol are mediated through interaction with two nuclear receptor proteins, ERα and ERβ, located in chromosomes 6q and 14q, respectively, which are encoded by two separate genes *ESR1* and *ESR2*, respectively. Both, ERα and ERβ show substantial homology in the DNA binding domain. Role of ERβ in breast cancer has not yet been determined. Hereafter, ERα will simply be referred to as ER
- The "classical" function of ER involves binding of 17βestradiol to ER located in the cell nucleus. This induces receptor dimerization, which binds to estrogen response elements (EREs) on many other genes, which are then indirectly regulated by estrogen and ERα
- ERE activated genes perform many important functions, including inhibition of apoptosis and stimulation of the cell cycle. There is cross talk with other mitogenic pathways (ras, raf, cyclin D1)
- Activation of estrogen target genes is accomplished through direct hormonal binding with the ER. This recruits protein regulators known as coactivators and repressors. Coregulators are responsible for chromatin remodeling to facilitate binding of RNA-polymerase. Histone acetylation, through acetyl transferases, correlates with a more actively transcribed state of chromatin regulation, whereas methylation favors more tightly coiled chromatin, which is less accessible to transcription and less gene expression



	Milk line	Placode	Mammary bud epithelium	Mammary bud mesenchyme
Secreted molecules and receptors	FGF10/FGFR2	WNTs	PTHLH	PTHR1
	WNT10b,6	FGFs	IGF1	BMP4/BMPR1A
	NRG3/ERBB2,4	BMP4	BMP4	
Transcription factors	TBX2,3	TBX2,3	LEF1	MSX2
	GLI3	LEF1	MSX1,2	
			TBX3	
			GLI3	

Fig. 6.3 Morphological stages in the embryonic development of the mammary gland in the mice: Around embryonic day 10 (E10) of mouse development, the milk line (*orange*) is defined by a slight thickening and stratification of the ectoderm (*gray*) as depicted here in this series of cross sections through the trunk. On E11.5, the milk line breaks up into individual placodes (*orange*) and the underlying mammary mesenchyme (*blue*) starts to condense. Over the following days, the placodes sink deeper into the dermis and the mammary mesenchyme becomes organized in concentric layers around the mammary bud (*orange*). Starting on E15.5, the mammary epithelium (*orange*) starts to proliferate at the tip and the primary sprouts pushes through the mammary mesenchyme towards the fat pad (*green*). On E18.5, the elongating duct has grown into the fat pad and has branched into a small ductal system. The cells of the mammary mesenchyme have formed the nipple, which is made of specialized epidermal cells (*purple*). *Lower*: The schematic diagram shows the position of the milk line, placodes, and mammary buds along the lateral body wall of early mouse embryos. Secreted molecules, receptors, and transcription factors that are important at the different stages are listed in the table below. At the mammary bud stage, proteins that are expressed in the epithelium and in the mesenchyme are listed separately. *BMP* bone morphogenic protein; *ERBB* erythroblastic leukemia viral oncogene homologue; *FGF* fibroblast growth factor; *FGFR1* fibroblast growth factor receptor; *GLI* Gli-Kruppel family member; *IGF* insulin growth factor; *IGFR* insulin growth factor receptor; *LEF* lymphoid enhancer-binding protein; *MSX* muscle segmentation homeobox; *NRG* neuroregulin; *PTHLH* parathyroid hormone-like hormone; *PTHR1* parathyroid hormone receptor; *TBX* T-box; *WNT* wingless-related MMTV integration site. (Reprinted with permission of Nature publishing group)

- ER status is highly predictive of clinical benefit from endocrine therapy in both adjuvant and metastatic disease settings. ER-positive tumors are more likely to respond to hormonal therapy, and have a better prognosis, when compared to ER− tumors
  - Harvey et al. showed in a cohort of 1,982 patients, using ligand binding assays (LBA) >3 fmol/mg and, retrospectively IHC (Allred Score >2 or 1–10% weakly positive cells), showed IHC to be a stronger predictor of disease-free survival (DFS) in patients receiving endocrine therapy when compared to LBA
  - Elledge et al., in a cohort of 205 patients, showed significant correlation of IHC ER and clinical response in patients with advanced metastatic disease (ER negative 25%, intermediate 46%, and high 66%)
- Accurate measurements of ER are of considerable importance, because it represents one of the strongest predictive factors of responsiveness to endocrine management. In some cases, endocrine therapy alone is an option, without additional cytotoxic therapy. About 70–80% of breast cancers are ER-positive and 20–30% are ER-negative. Only 70% of ER-positive tumors show clinical response to estrogen manipulation, but measuring ER expression alone is insufficient to distinguish responders from nonresponders. A significant fraction of patients with ER-positive disease eventually develop resistance to endocrine therapy
- Clinical progression of the ER-positive breast cancer typically correlates with hormone resistance. Loss of response and decreased ER expression are associated with a more aggressive clinical course. Epigenetic alterations of the ER promoter, including methylation of *ESR1* gene, are thought to be important events in the development of ER-negative breast cancers
- In the last decade, prospective randomized clinical trials have shown the superiority of aromatase inhibitors over tamoxifen in postmenopausal receptor-positive women
- Tamoxifen is a partial agonist (both antagonistic and agonistic effects) of the ER receptor, and induces dimerization and nuclear translocation and is designated as a selective ER modulator (SERM)
- Fulvestrant directly binds to ER monomers, inhibits dimerization, and suppresses activation, thereby functioning as a pure antiestrogen. Its benefits have been demonstrated in the metastatic setting, and ongoing trials are underway in the adjuvant setting
- Anastrozole, letrozole, and exemestane are aromatase inhibitors (AI) which block the conversion of adrenally produced precursor compounds to estrogenic molecules. Recent trials also showed the benefits of estrogen deprivation persist for many years even after completion of the initial hormonal therapy in reducing both unilateral and contralateral breast cancers
- Recently, the Women's health Initiative Estrogen-Alone trial, analyzed, prospectively, the use of equine-conjugated estrogen (CEE) among patients with prior hysterectomy. The trial was stopped earlier and showed a decreased risk of breast cancer in the treatment group
- Progesterone has an essential role in regulating breast maturation. A clear role in carcinogenesis has been shown in animal models, particularly in respect to induction, maintenance, and progression of the neoplastic phenotype. An increased risk of breast cancer is documented in long-term users of progestin-only containing hormone-replacement therapy (HRT) regimens
- ER is important for regulating PR expression. Colocalization studies show that PR expressing cells also express ER. In fact, PR expression is regarded as a marker of an intact ER axis. However, discrepancies exist: the relative risk of disease recurrence is higher in patients with ER+/PR– cancers, compared to ER+/PR+ tumors
- About 60% of breast cancers express PR. This expression is regarded as a marker of intact ER function. PR receptor is also nuclear. Progesterone effects are mediated through the intracellular proteins PRA and PRB. Both are coded from the same gene using two distinct translation initiation sites

- Expression of PR in breast cancer is also associated with higher responsiveness to endocrine therapy. The majority of HER2-positive cancers are PR-negative, suggesting that nuclear ERα may be nonfunctional in these cases. However, membrane ER appears to remain functional and promotes tumor cell proliferation in cooperation with overexpressed *HER2*. In this setting, tamoxifen (as a partial agonist) may theoretically help induce cell proliferation. In this setting, AI will remain beneficial. A role for highly quantitative assessment of PR might be helpful in more precisely predicting response in patients with ER-positive/HER2-positive tumors
- Most testing for ER and PR today is done using IHC. However, errors have been problematical when using IHC. For example, the United Kingdom National External Quality Assessment Service (UK NEQAS) evaluated the frequency of hormone-receptor-positive cancers in more than 7,000 patients, highlighting significant variation in ER and PR positivity rates. Similar results were obtained by the Royal College of Pathologists of Australasia ($n=8{,}000$ patients). Approximately one-third of 1,023 ER tests performed on patients, in Canada, between 1997 and 2005 were scored falsely negative, which was revealed by retesting in an expert central laboratory in Ontario. More than 100 of these patients have since died and a class action lawsuit ensued claiming negligence in ER testing and failure to provide Tamoxifen to these patients. Investigation into the matter identified many causes of false negative IHC results, including: poor sample fixation, improper staining procedures, and improper interpretation:
  - The International Breast Cancer Study Group (IBCSG) conducted a series of studies comparing chemo and endocrine treatment to endocrine treatment alone in years before the establishment of IHC testing: Most studies of ER testing used LBA or ELISA. They compared with results obtained after the primary tumor blocks were collected and reanalyzed in a single central lab using IHC. Discordant ER results between institutional and central results were 16% (ER+) and 24% (ER−) for specimens from premenopausal women, and 9% (ER+) and 24% (ER−) from postmenopausal women. Overall concordance rate was 82 and 88% for pre- and postmenopausal women, respectively
  - In the ECOG 2197 trial, 11% of local ER− tests were scored positive on central testing, with an overall concordance rate of 90%
  - In the ALTTO trial (5,000 patients from countries worldwide), so far, 4.3% of tumors that tested ER+ in local laboratories were found to be negative (false-positive) on central review. More than 20% of tumors exhibited at least some expression of ER (false-negative) on central review

Guidelines for Estrogen Receptor and Progesterone Receptor Testing by Immunohistochemistry

- In an effort to improve the quality of testing for ER and PR by IHC, the American Society of Oncologists (ASCO) and College of American Pathologists (CAP) jointly developed and recently published guidelines for pathologists to follow (Fig. 6.4 and 6.5). Compliance with the guidelines is now mandatory for laboratories in the US to receive CAP accreditation
- Immunohistochemistry on FFPE tissue replaced LBAs for testing in the late 1980s. Harvey et al. compared the predictive abilities of LBA and IHC using the 6F11 antibody in a large cohort of patients with newly diagnosed breast cancer. This cohort received a variety of types of adjuvant therapy that ranged from none to endocrine alone, chemotherapy alone, or a combination of the above. Receptor status was scored as the sum of the proportion and average intensity scores of positive staining tumor cells (Allred Score on a scale ranging from 0 to 8). On the basis of clinical outcome in patients with adjuvant endocrine therapy, patients with Allred Score >3 (corresponding

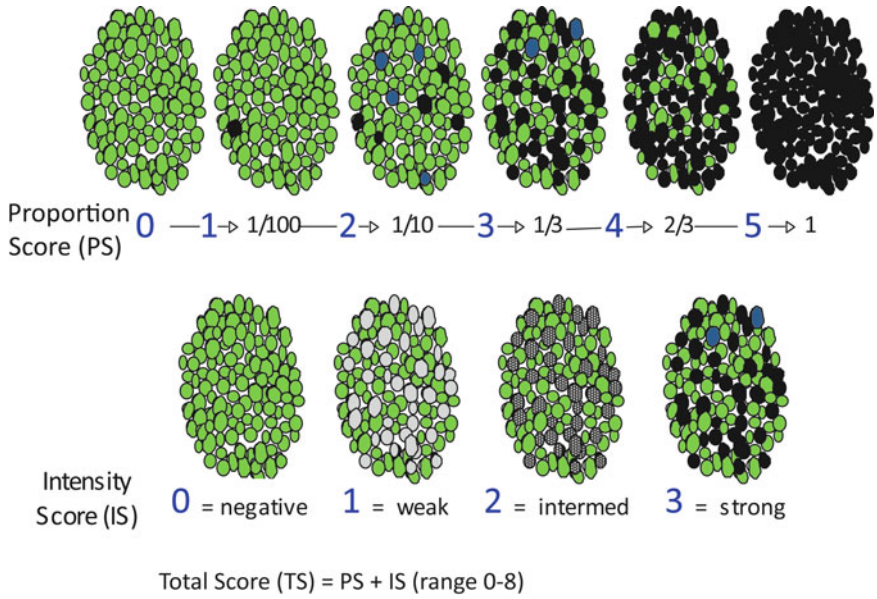


Fig. 6.4 Algorithm for scoring biomarkers (ER, PR) according to recent ASCO guidelines. Allred Score. A combination of number of cells (Proportion Score) and intensity of staining (Intensity Score) is used. (Adapted from Allred et al.)

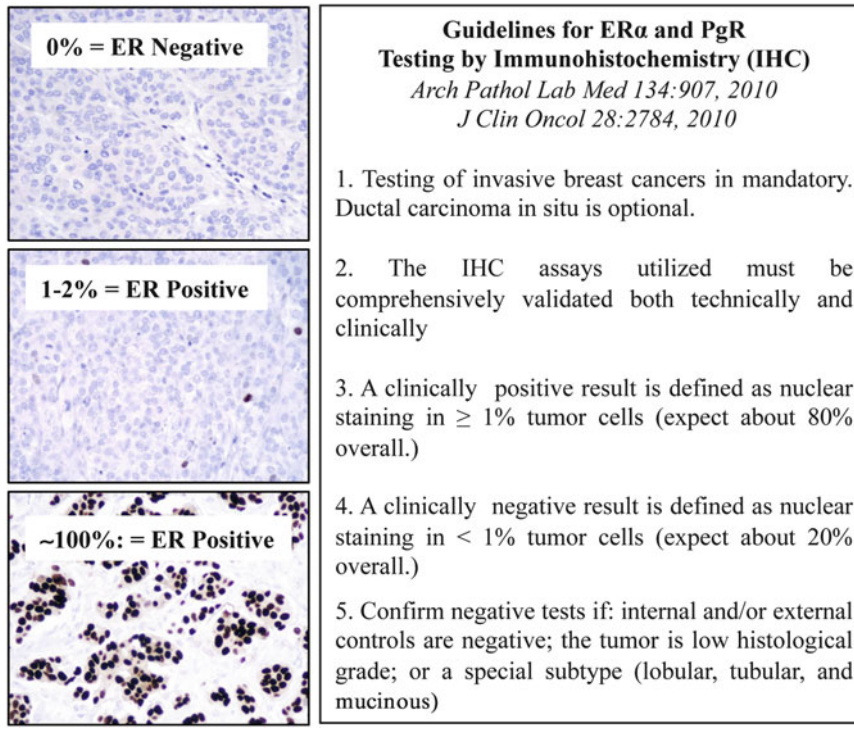


Fig. 6.5 Schematic example of ER interpretation

to as few as 1–10% positive cells) had a substantially and statistically significant better prognosis than patients with scores less than 3 (<1% positive cells). The predictive ability of IHC was superior to LBA previously performed in the same tumors

- There is no gold standard available for IHC assays of ER and PR. A relevant standard would be any assay whose specific preanalytic and analytic components conformed to assays whose results have been validated against clinical benefit from endocrine therapy (clinical validation). Several assays meet this criteria such as the methods described in the publication by Harvey et al. and Mohsin et al., the FDA 510(k)-cleared ER/PR pharmDx assay kit (Dako, Glostrup, Denmark)
- ER status can also be determined at the RNA level. The Oncotype DX® Assay measures RNA expression of 21 genes to determine a recurrence score (RS). ER and PR are among the most prevalent genes in the signature. Comparison between measures of the ER/PR protein by IHC and of mRNA by RT-PCR showed a discordance rate of 9% and 12%, respectively. There are no published correlations of the individual measures of ER and PR mRNA from the 21-gene signature with the clinical outcome
- A laboratory that performs ER testing should validate its proposed or existing assay against one of the clinically validated assays and demonstrate acceptable concordance. To be considered acceptable, the results of the assay must be initially 90% concordant with those of the clinically validated assay for the ER-positive and PR-positive categories, and 95% concordant for the ER-negative or PR-negative categories
- The cutoff from distinguishing a "positive" from "negative" cases should be ≥1% ER+ positive tumor cells. Patients whose breast tumors show at least 1% ER+ cells are candidates for endocrine therapy and those with less are not. Percentage of stained tumor cells provides valuable predictive and prognostic information to inform treatment strategies
- Eight studies described the relationship between hormone-receptor levels and patient outcomes. Overall survival, DFS, recurrence/relapse-free survival, 5-year survival, time to treatment failure, response to endocrine therapy, and time to recurrence were positively related to ER levels
- PR status provides additional predictive value independent of ER values, especially among premenopausal women. Its predictive value has been demonstrated in retrospective studies using 1% as cutoff point. Among patients who received adjuvant endocrine therapy, the best cutoff for both DFS ($P=0.0021$) and OS ($P=0.0014$) was a total PR Allred Score >2, which corresponds to greater than 1% of carcinoma cells exhibiting weakly positive staining. In patients with metastatic breast cancer who received first-line endocrine therapy on relapse, a correlation with PR status and response to endocrine therapy was found at a 1% staining threshold ($P=0.044$) or response to tamoxifen at 10% ($P=0.021$). Patients with carcinomas >1% PR staining had a better survival after relapse ($P=0.0008$)
- Reporting results for ER, PR, and HER2: The percentage and proportion of tumor cells staining positively should be recorded and reported. All tumor areas of the tissue section on the slide should be evaluated. This can be achieved manually by counting cells or through image analysis
- The intensity of the staining should be recorded and reported as weak, moderate, or strong. This measurement should represent an estimate of the average staining of the intensity of the positively stained tumor cells on the entire section relative to the intensity of the positive controls run on the same batch. A cutoff of a minimum of 1% of the tumor cells positive for ER/PR for a specimen is considered to be positive. The term equivocal must not be used
- Less than 1% of the tumor cells positive for ER/PR for a specimen is considered to be negative. Such patients do not receive meaningful benefit from endocrine therapy

- Any specimen lacking intrinsic elements (normal breast epithelium) that is negative on ER and/or PR assay should be repeated using another tumor block or another specimen, and reported as not interpretable rather than as negative
- "Not interpretable" receptor results refer to samples that did not conform to preanalytic specifications of the guidelines, were processed using procedures that did not conform to guideline specifications of the lab operating procedures, or the assay used to analyze the specimen was not validated and controlled as specific in the guideline. Examples of circumstances leading to not interpretable results include testing of needle biopsies or cytology samples fixed in alcohol, use of fixatives other than 10% NBF, biopsies fixed for intervals shorter than 6 h or longer than 72 h, samples where fixation was delayed more than 1 h, samples with prior decalcification, and samples without internal or external controls
- Negative ER and PR interpretations in tumors that characteristically have an ER+ phenotype (e.g., lobular, tubular, and mucinous carcinomas) should be confirmed by retesting
- ER and PR should be documented in all newly diagnosed breast cancers. Recurrences should also always be tested to exclude prior false negatives, and to document changes in biologic behavior. In the routine practice, DCIS is also commonly tested for ER and PR based on the NSABP-24 clinical trials. The trial compared placebo versus tamoxifen after lumpectomy and radiation. There was a significant reduction (40–50%) in subsequent breast cancer (ipsilateral and contralateral) restricted to patients with DCIS ER+ at 10 years followup

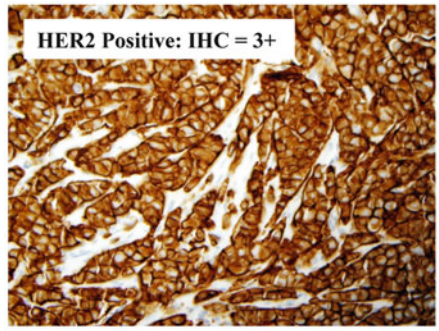
Human Epidermal Growth Factor Receptor 2 Gene: Molecular and Clinical Aspects

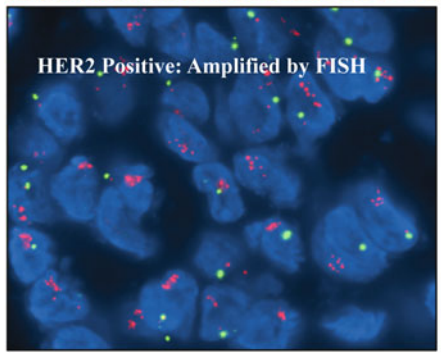
- The human epidermal growth factor receptor 2 gene, more commonly referred to as *HER2*, is amplified in 15–25% of human breast cancers. *HER2* amplification and overexpression are highly correlated, which are significantly associated with aggressive disease (i.e., poor prognostic factors), and are the molecular targets for specific therapies, such as trastuzumab
- *HER2* is a protooncogene located on chromosome 17. It encodes a tyrosine–kinase receptor residing in the surface membrane of breast epithelial cells. It forms complexes with similar proteins (erbB1, erbB3, and erbB4) and acts as receptors for several ligands, such as EGF, heregulin, and amphiregulin. It regulates many normal cell functions, including proliferation, survival, and apoptosis
- The overall relationship between HER2 and clinical outcome is complex and varies with the clinical setting. A weak but significant association between poor outcome and a positive HER2 (overexpression or amplification) in patients receiving no additional therapy after initial surgery is seen. But this only represents a small fraction of patients today. The majority of patients typically receive some form of adjuvant treatment. Some studies have shown that HER2+ breast cancers are resistant to certain types of cytotoxic chemotherapy (e.g., the combination of cyclophosphamide, methotrexate, and 5-fluorouracil) but sensitive to others (e.g., anthracyclines and taxanes). In general, it is accepted that HER2+ cancers appear to be associated with relative, but no absolute, resistance to endocrine therapies in general. However, this issue remains very controversial. The most promising and useful findings are based on recent studies showing that HER2+ cancers respond favorably to new antibody-based therapies, targeting specifically the HER2 protein, such as trastuzumab. Although this therapy was originally demonstrated effective in patients with metastatic disease, more recent clinical trials have shown significant benefits in the adjuvant setting for patients with less advanced disease. The NSABP-B31 clinical trial, which randomized patients with HER2+ cancer to adjuvant chemotherapy +/− trastuzumab, showed a 52% improvement in disease-free survival with the monoclonal antibody

- A long and persistent controversy in the evaluation of the HER2 status by protein expression through IHC, or gene amplification by FISH exists. However, many studies have shown that, when properly performed, a very strong correlation between the two methods exists, and they are equivalent and complimentary in the clinical practice
  - Owens et al. observed a similar frequency of HER2 amplified cases by IHC (20%) among 116,736 specimens and FISH (22%) among 6,556 specimens
  - Most clinical trials using trastuzumab enroll patients with IHC positive, or reflex FISH positive, or ISH alone
- In general, approximately 70% of breast cancers show little or no protein overexpression, a normal gene copy number, and do not respond to trastuzumab. Roughly 15% show low to intermediate levels of protein expression, and the gene is amplified in nearly a third of those cases. There is still uncertainty of how well these patients respond to the drug. The remaining 15% of cases show very strong membrane staining, indicating high levels of protein expression and the gene is nearly always amplified. This is the population who shows best response to trastuzumab

Guidelines for HER2 Testing in Breast Cancer

- ASCO and the CAP jointly developed and published guidelines to improve the quality of HER2 testing (Fig. 6.6)
- A positive HER2 test is defined as a result of 3+ surface protein expression (formed as uniform intense membrane staining of >30% of invasive tumor cells) or FISH result of amplified *HER2* gene copy number (average of >6 copies/nucleus for test systems without internal control probe) or *HER2/CEP17* ratio of more than 2.2, where CEP17 is a centromeric probe for chromosome 17 on which the HER2 gene resides
- Originally, FISH testing results were reported as either positive or negative, but an intermediate range (referred as equivocal range) has since been described and its clinical significance remains unclear. Much of the confusion using this term comes from the need to define the need for trastuzumab treatment. There is also significant variation in the intermediate (equivocal) ranges for both the IHC and FISH assays. The equivocal range for IHC consists of samples scored 2+, which includes up to 15% of samples. An equivocal result (2+) is complete membrane staining that is either nonuniform or weak in intensity but with obvious circumferential distribution in at least 10% of cells. Some, but not all of these samples may have *HER2* gene amplification and require additional testing to define the true HER2 status. The equivocal range for FISH assays is defined as *HER2/CEP17* ratios from 1.8 to 2.2 or average gene copy numbers between 4.0 and 6.0 for systems without an internal control probe. About 3% of patients have ratios of 2.0–2.2 and were previously included in treatment arms with trastuzumab. Polysomy 17 is a vague term, seen in up to 8% of tumors. If polysomy 17 is defined as three or more copies of CEP17, most are not associated with protein or mRNA overexpression
- Discordant results (IHC3+/FISH– or IHC<3+/FISH+) have been documented in approximately 4% of cases. The significance of this is unclear. Equivocal results of a single test require additional action, which should be specified in the report. Equivocal results by IHC should follow confirmatory FISH analysis. Counting additional cells or repeating the test confirms equivocal FISH results. If the results remain equivocal, confirmatory IHC is recommended
- A negative HER2 test is defined as either an IHC result of 0 or 1+ for cellular membrane protein expression (no staining or weak, incomplete membrane staining in any proportion of tumor cells), or a FISH result showing *HER2/CEP17* ratio of less than 1.8 or an average of fewer than four copies of *HER2* gene per nucleus for systems without an internal control probe





Guidelines for HER2 Testing
Arch Pathol Lab Med 131:18, 2007
J Clin Oncol 25:118, 2007

1. Testing of invasive breast cancers is mandatory.

2. Either immunohistochemistry (IHC) or fluorescent in situ hybridization (FISH) assays may be utilized, providing that they have been comprehensively validated technically and clinically.

3. Positive by IHC is defined as strong circumferential membrane staining in >30% tumor cells (referred to as 3+; expect about 15% of tumors overall). Absent (0) or weak (1+) intensity staining are considered as negative results (about 70% overall). Staining in between is considered equivocal (2+) and the tumor must be retested for confirmation (usually by FISH).

4. Positive by FISH is defined as a gene-to-chromosome ratio > 2.2, or gene copy number > 6 (about 15% overall). A ratio of < 1.8 is negative (about 70% overall). A ratio in between is equivocal (about 15% overall) and the tumor must be retested and confirmed (by IHC or counting more cells by FISH).

Fig. 6.6 Algorithm for scoring HER2 according to recent ASCO guidelines

- The ASCO/CAP guidelines establish that, in order to classify a test as positive or negative, the laboratory must have performed concordance testing with a validated FISH assay and confirmed that only 5% or less of samples classified as either + or − disagree with the validated assay on an ongoing basis. Equivocal cases are not expected to be 95% concordant, but rather subjected to a confirmatory test

Recent Advances in the Molecular Pathology of Breast Cancer of Clinical Significance

Multigene Prognostic Indices

- Oncotype DX® is a prognostic test measuring the RNA expression of 21 genes, which provides a recurrence score (RS; range 0–100) using FFPE tumor samples. The genes include proliferation markers (*Ki67, survivin, cyclin D1*), invasion-related (*MMP11, cathepsin*), *HER2*, ER, PR, and others (*GSTM1, CD68, BCL2*), as well as five housekeeping genes used to normalize expression overall. The RS quantifies the likelihood of disease recurrence based on studies in women with early stage hormone estrogen receptor (ER) positive only breast cancer, and assesses the likely benefit from certain types of chemotherapy. Scores are reported as: low (<18), intermediate (18–31), or high (>31) relative to risk of recurrence. Typically, patients in the high risk receive chemotherapy and those in the low risk do not. Studies have demonstrated that treatment is modified in 31% of patients who are tested by Oncotype DX®, including omission of presumed unnecessary chemotherapy in 22%. Based on these findings, it is estimated that the cost of gene expression against the relative costs of ER, PR, and HER2 are likely to result in an overall cost saving, as well as reduced toxicity and quality of life

improvements for patients. Recently, the test has also shown similar prognostic and predictive significance in women with receptor-positive node-positive received adjuvant treatment with the aromatase inhibitor anastrozole, and in cancer patients receiving neoadjuvant hormonal therapy and chemotherapy. There is an important ongoing phase III clinical trial, referred to as the TAILORx study, designed to help optimize the use of adjuvant endocrine and chemotherapy in patients with receptor-positive breast cancer. Based on their recurrence score, women will be assigned to three different treatment groups: women with a recurrence score higher than 25 will receive chemotherapy plus hormonal therapy (the standard of care); women with a recurrence score lower than 11 will receive hormonal therapy alone; and women with a recurrence score of 11–25 will be randomly assigned to receive adjuvant hormonal therapy, with or without chemotherapy. The study is primarily designed to evaluate the effect of chemotherapy on those with a recurrence score of 11–25. Because the degree of benefit of chemotherapy for women with recurrence scores between 11 and 25 is uncertain, strong preliminary evidence suggests that may only require endocrine therapy, which would be an important benefit

- The Mammaprint®: 70-gene prognostic index was validated as clinically useful in studies of younger women with node-negative breast cancer by classifying them into low risk and high risk for disease recurrence. It requires frozen tumor samples. Genes involved in the regulation of cell cycle, invasion, and angiogenesis heavily weight it. Genes of interest do not include known prognostic markers such as ER, PR, and HER2. High risk patients are most likely to benefit from cytotoxic chemotherapy. In contrast, the low risk group typically responds very well to endocrine therapy without chemotherapy. The prospective validation of the MammaPrint® signature's prognostic value is currently ongoing through the Microarray in Node-Negative Disease May Avoid Chemotherapy (MINDACT) trial. This trial opened in February 2007 as has enrolled over 6,000 patients from five European countries. It assesses all patients by the standard clinicopathologic prognostic factors included in adjuvant setting and by the 70-gene signature assay. If both traditional and molecular assays predict a high risk status, the patient receives adjuvant cytotoxic chemotherapy and also hormonal therapy if ER positive. If both assays indicate a low risk, no chemotherapy is given and ER-positive patients are given adjuvant hormonal therapy only. When there is discordance between the traditional clinicopathologic prognostic factor prediction of risk and the 70-gene signature prediction of risk, the patients are randomized to receive treatment based on either the genomic or the clinical prediction results. The primary goal of the study is to confirm that breast cancer patients with a "low risk" molecular prognosis by MammaPrint® and "high risk" clinical prognosis can be safely spared chemotherapy without affecting distant metastases-free survival (DMFS)
- PAM50 assay: was developed to efficiently determine intrinsic molecular subtypes based on evaluating 50 carefully selected genes using next generation sequencing and FFPE tissue samples. It is currently performed in a commercial reference laboratory, but an instrument dedicated to perform this will be available to pathology laboratories in the future. The PAM50 test provides a risk of relapse score (ROR) initially based on studies of patients with node-negative breast cancer who did not receive adjuvant systemic therapy. The ability of ROR to predict prognosis has recently been confirmed as useful in an independent set of 786 patients with ER+ treated only with tamoxifen. In these studies, ROR was a better predictor than standard clinicopathologic variables, including Ki67, PR, and histological grade. Most recently, PAM50 outperformed OncotypeDX® for predicting response to endocrine therapy in a large prospective clinical trial of receptor-positive node-negative patients

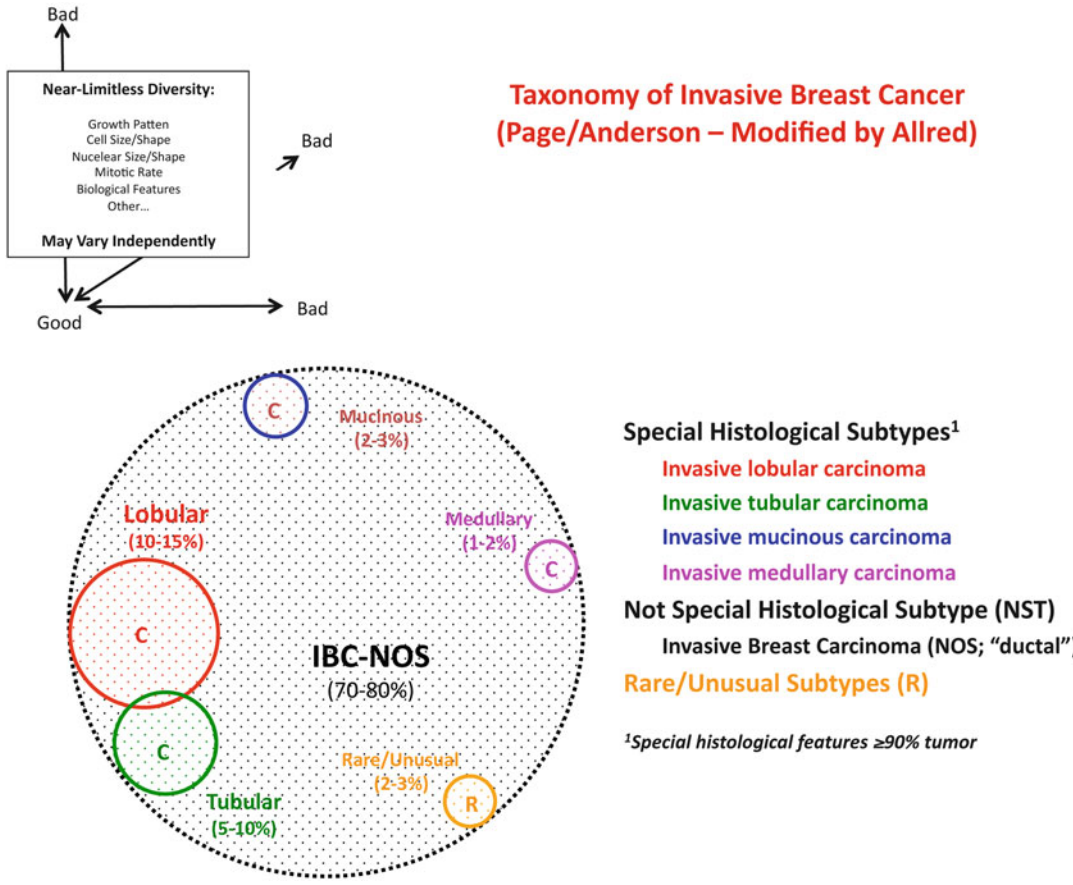


Fig. 6.7 Taxonomy of breast cancer. WHO classification of common histologic subtypes

- MapQuant Dx® genomic grade: is a predictor test derived by identifying 97 differentially expressed genes from grade 1 and 3 breast cancers using a training set of 64 ER+ tumors. Most genes are cell cycle regulators and proliferation. Genomic grade index (GGI) was strongly associated with risk of recurrence among patients with grade 2 tumors. It requires fresh tissue, similar to Mammaprint
- Breast cancer index (BCI): provides assessment of likelihood of distant recurrence in patients with ER+, node-negative breast cancer treated with endocrine therapy (primarily tamoxifen). BCI was developed from a combination of two indices: HOXB13:IL17BR and a proliferation related five-gene molecular grade index. Technically, it involves using a qRT-PCR assay with FFPE tissue samples
- The clinical use of Mammaprint®, Oncotype DX®, BCI, PAM50 assays have all been proven most useful in studies of patients with receptor-positive node-negative breast cancer, which are highly enriched with luminal A molecular subtypes, which may explain why the prognostic ability of these different gene expression-based assays is similar, as most of them are differentiating luminal A from all other subtypes (Figs. 6.7 and 6.8)

Intrinsic Molecular Subtypes of Breast Cancer

- Understanding the more recent advances in the molecular biology of breast carcinogenesis, imply acknowledging the major contribution of

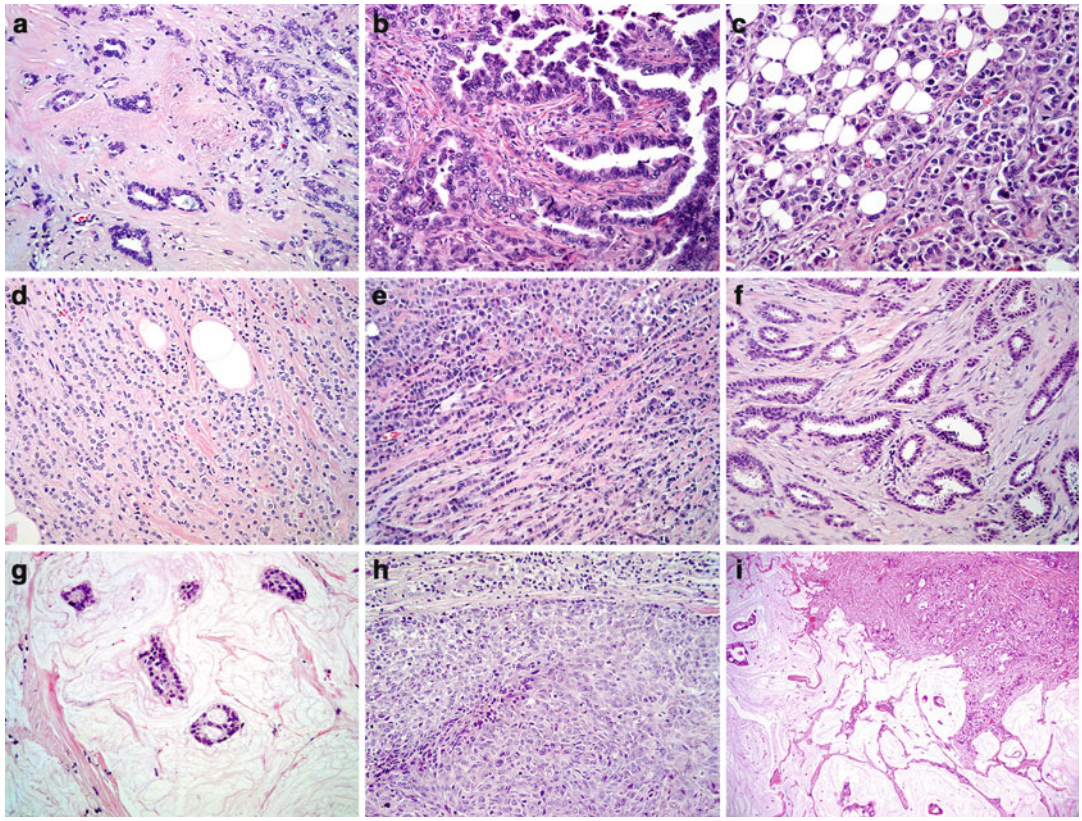


Fig. 6.8 Breast cancer pathology, common histologic subtypes. Grading invasive carcinomas depend on the degree of tubular formation, nuclear features, and mitotic index. Invasive ductal carcinoma of no special type. 8a-Grade 1; 8b-Grade 2; 8c-Grade 3. 8d-8i, common histologic types. 8d and 8e: invasive lobular carcinoma; 8f: invasive tubular carcinoma; 8g: invasive mucinous carcinoma; 8h: invasive medullary carcinoma; 8i: invasive ductal carcinoma with mucinous features

Perou et al. in the description of the molecular intrinsic subtypes of breast carcinomas. This work represents the first molecular classification of tumors, not considering the histology but a description of gene expression profiles of different breast tumors

- Four molecular subtypes were originally described: luminal, normal breast-like, HER2, and basal like. Subsequently luminals were further subdivided into Luminal A and Luminal B
- • Luminal tumors are reminiscent of "normal luminal epithelial cells," including CK8/18+. Lum A are ER+ and enriched with genes associated with active ER pathway, low levels of proliferation related genes, low histological grade, and generally good prognosis. The Lum B tumors are typically higher grade, with high proliferation indexes, and worse outcome, and a significant proportion are HER2+. Recent data show no good separation between Lum A and Lum B based on proliferation
- The normal breast-like subtype has gene expression profiles similar to fibroadenomas and normal breast enriched in adipose tissue genes. They are relatively poorly characterized and their prognostic significance is unclear. Recent studies suggest that the normal breast-like group may be an artifact caused by contamination of samples with normal tissue
- The HER2+ subtype shows amplification or 3+ reactivity by IHC, and expresses many other genes associated with the HER2 pathway.

However, a good number of *HER2* amplified, ER+ cancers fall into Lum B category

- The basal subtype expresses genes found in normal basal/MECs of breast, such as *CK5, CK14, p-cadherin, caveolins 1–2, CD44*, and *EGFR*. A minority has *EGFR* amplification. However, unlike MECs, they also express certain proteins characteristic of LECs, such as CK8, CK18, and KIT. Basal-like carcinomas are usually high histological grade tumors with high proliferation, necrosis, pushing borders, and lymphocytic infiltrate. Histological subtypes commonly seen in this category include medullary or metaplastic carcinomas. The basal-like subtype more commonly happen in younger individuals, often of African–American or Hispanic decent. The tumors usually show high initial response to cytotoxic chemotherapy, although the majority relapses and overall prognosis is very poor. These features are similar to those seen in tumors of patients with *BRCA1* mutation and the BRCA1 pathway is dysfunctional in basal-like cancers
- Three new ER-negative molecular subtypes have recently been described: One, referred to as "Molecular apocrine," is similar to HER2 subtype but shows activation of androgen receptor signaling; another, referred to "Interferon subtype," are characterized STAT1; and the third are referred to as the "claudin-low" group, which typically demonstrate a cancer-stem cell like phenotype
- Recently, several studies have questioned whether intrinsic subtyping is reproducible or stable, and whether it has any useful clinical significance
- The relationship of intrinsic molecular subtypes to special histological subtypes of breast cancer: Some studies, mainly using microarray-based technology, have shown that at the transcriptional level, tubular, mucinous, and lobular subtypes are more homogeneous than invasive ductal carcinomas of no special type (IDC/NST). Tubular, mucinous, and neuroendocrine carcinomas are typically included in the luminal phenotype. Adenocystic, medullary, and metaplastic are basal-like in agreement with previous studies
- The use of IHC has recently been advocated as a surrogate to microarray analysis to define the intrinsic molecular subtypes (Fig. 6.11): Expression by IHC of ER, PR, and luminal CKs (CK8 and CK18), lack of HER2 overexpression, and low Ki67 are typical of Lum A. Expression of ER, PR, and luminal CKs, and HER2 overexpression are seen in Lum B. Absence of ER and PR, and HER2, and expression of basal CKs (CK5/6) define basal-like tumors
- In the neoadjuvant settings, pathologic complete response (pCR) has been used to determine response to chemotherapy. pCR is only seen in 20–30% of patients (with use of standard anthracycline and taxane-based chemotherapy): Different rates have been shown across the different molecular subtypes: rates are 7% for Lum A, 17% for Lum B, 36% for HER2, and 43% for basal-like. This is one of the few scenarios where the use of molecular subtypes is advocated to translate into clinical practice. It is important to understand that molecular subtypes do not add much additional information of prognostic significance compared to the current standards of histologic subtypes and pathologic grading
- Even though the molecular classification has been one of the greatest advances in breast cancer in the last two decades, differences in molecular aspects of common histologic subtypes have been also recognized. Here are some examples: Medullary carcinomas show a prominent T helper cell immune response. Adenoid cystic carcinomas of the breast show a characteristic translocation *t*(6;9), which creates a *MYB–NFIB* fusion transcript. Secretory carcinomas also have an associated translocation, *t*(12;15) with the conformation of a *ETV6–NTRK3* fusion transcript. Micropapillary carcinomas have a high rate of lymph node metastasis and are typically included in the luminal B subtype, but a distinct set of gene clusters on their own, including high rate *FGFR1* amplification. Metaplastic breast cancers are a mixture of adenocarcinoma with metaplastic elements, homologous (squamous and spindle metaplasia) or heterologous (chondroid, osteoid, skeletal muscle). They

are typically associated with *PI3K/AKT* mutations—over 90% are HER2 and ER negative, and typically show a basal-like immunophenotype. A dysfunctional BRCA1 pathway is seen with over 60% of metaplastic carcinomas, which is caused by methylation silencing of the *BRCA1* gene promoter. In addition, a mouse model with *BRCA1* inactivation and wild-type allele of *TP53* show classical morphologic features of metaplastic carcinomas, including HER2 and basal markers (CK14 and EGFR), as well as activation of WNT pathway (Figs. 6.9, 6.10, and 6.11)

Important Somatic Mutations in Breast Cancer

- *TP53* is mutated in up to 30% of sporadic breast cancers, as well as many other types of cancers. The gene is located on chr 17 and encodes a nuclear transcription factor normally involved in cellular pathways activated in response to stress by inhibiting the proliferation, and inducing apoptosis, of cell damaged in a variety of ways. P53 acts as a transcriptional activator of genes involved in inhibition of the cell cycle, blood vessel formation, stimulation of apoptosis, and promotion of DNA repair. Currently, 2,500 different inactivating *TP53* mutations have been described in breast cancer. About 75% are single nucleotide substitutions leading to substitution of a single amino acid, and the remaining 25% are insertions, deletions, and nonsense mutations. Mutations in one allele are associated with inactivation of the other one by loss of heterozygosity (LOH) in most affected breast cancers. Mutation of the gene often correlates with increased nuclear p53 expression by IHC, which can be used as an easy surrogate assay in certain situations. Somatic mutations of *TP53* occur in IBCs and DCIS. In both settings, they are associated with increased tumor size and grade, as well as axillary metastasis and the rate of *TP53* mutations is very high in *BRCA1/BRAC2* carriers. The presence of *TP53* mutations is associated with poor prognosis: shorter DFS and OS in both node-negative and node-positive cancers. However, one study has shown an advantage in survival in node-negative breast cancer with mutated *TP53* treated with XRT compared to node-negative with WT *TP53*
- *ESR1* mutations: ERα has been reported as mutated and amplified in low percentage of breast cancers. Those with an ER A86V mutation are associated with lower activity of the receptor. The ER K303R mutation makes the receptor hypersensitive to activation by estrogen, which may promote tumor progression. An ER 437 stop codon mutation has been identified in metastatic breast cancers, and may be important in promoting metastatic spread, although the mutation is very rare
- Gene copy number alterations (referred to as allelic imbalance): AI is very common in breast cancers, occurring in as many as 50%. Gene amplification is a pathologic change commonly associated with increased mRNA transcription and protein expression of affected genes. Gene deletions are associated with loss of expression and function. Amplification of several regions in the breast cancer genome contains genes coding for oncogenes. For example, the chromosome 17q12 amplicon contains the *HER2* gene, the 8p24 amplicon the *MYC* gene, the 11q13 amplicon the *CCND1* gene, and the 6p11 the *ESR1* gene
  - Amplification of *HER2* is common in breast cancer and was discussed in detail above
  - Amplification of *ESR1* on chromosome 6p occurs in 5–20% of breast cancer, it is associated with increased ER expression, and it appears to increase responsiveness to tamoxifen therapy—so determining this feature may help optimize the use of endocrine therapy
  - 8q24 *MYC* on chromosome 8q24 is frequently amplified. MYC regulates cell growth and proliferation, and amplification is associated with higher histological grade, high proliferation rate, early recurrence, and death. Coamplification of *MYC* and *HER2* is very common, and trastuzumab is

Gene expression patterns of 85 experimental samples representing 78 carcinomas, three benign tumors, and four normal tissues, analyzed by hierarchical clustering using the 476 cDNA intrinsic clone set.

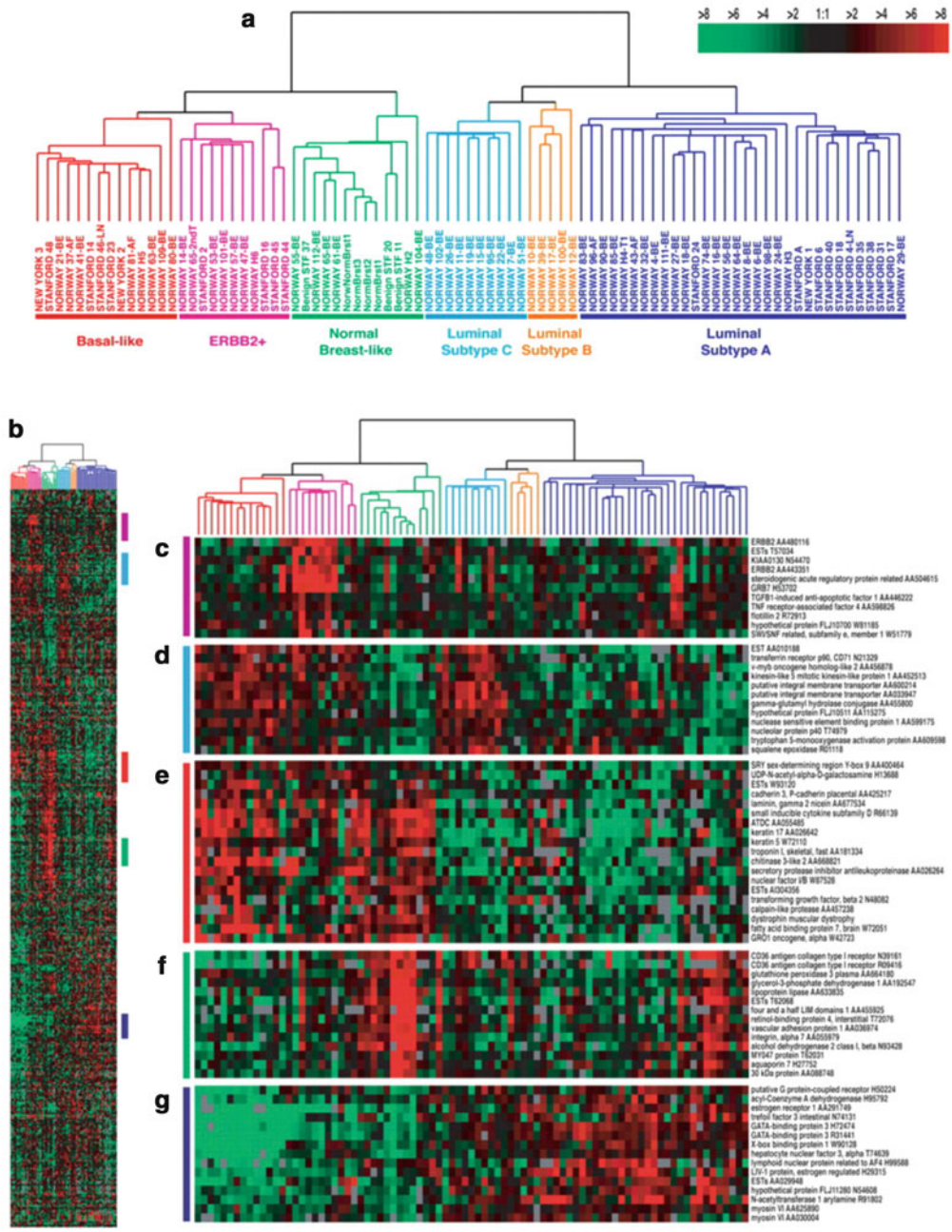


Fig. 6.9 Breast cancer molecular intrinsic subtypes. Gene expression patterns of 85 experimental samples representing 78 carcinomas, 3 benign tumors, and 4 normal tissues, analyzed by hierarchical clustering using the 476 cDNA intrinsic clone set. (**a**) The tumor specimens were divided into five (or six) subtypes based on differences in gene expression. The cluster dendrogram showing the five (or six) subtypes of tumors are colored as: luminal subtype A, *dark blue*; luminal subtype B, *yellow*; luminal subtype C, *light blue*; normal breast-like, *green*; basal-like, *red*; and ERBB2+, *pink*. (**b**) The full cluster diagram scaled down. The *colored bars* on the right represent the *inserts* presented in **c–g**. (**c**) ERBB2 amplicon cluster. (**d**) Novel unknown cluster. (**e**) Basal epithelial cell-enriched cluster. (**f**) Normal breast-like cluster. (**g**) Luminal epithelial gene cluster containing ER. (Copyright 2001 National Academy of Sciences, USA, with permission)

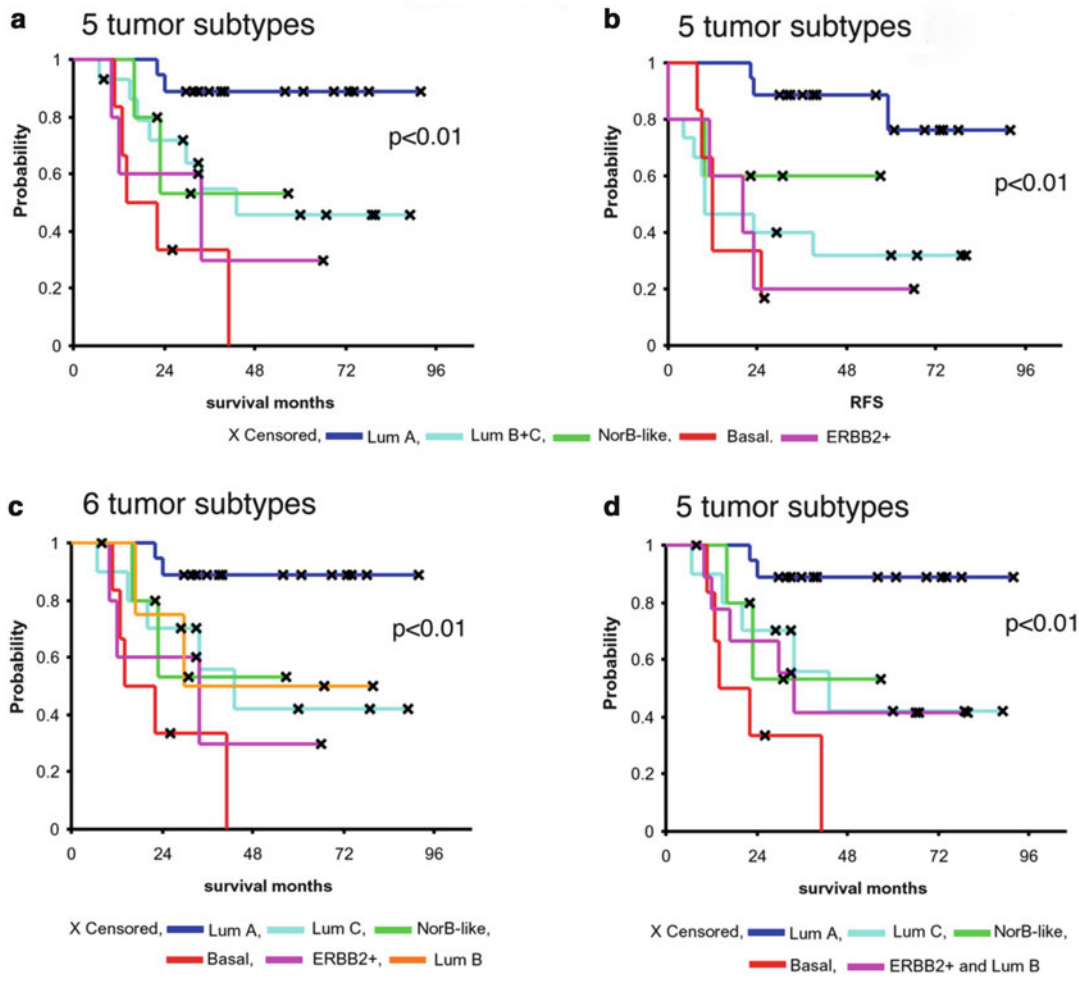


Fig. 6.10 Overall and relapse-free survival analysis of 49 breast cancer patients, uniformly treated in a prospective study, based on different gene expression classification. Overall and relapse-free survival analysis of the 49 breast cancer patients, uniformly treated in a prospective study, based on different gene expression classification. (**a**) Overall survival and (**b**) relapse-free survival for the five expression-based tumor subtypes based on the classification presented in Fig. 6.9 (luminals B and C were considered one group). (**c**) Overall survival estimated for the six-subtype classification with the three different luminal subtypes presented in Fig. 6.1. (**d**) Overall survival based on the five-subtype classification. (Copyright 2001 National Academy of Sciences, USA, with permission)

associated with improved outcome when coamplification exists compared to tumors with amplified *HER2* alone

– *CCND1* on chromosome 11q13 encodes a cell cycle regulatory protein that plays an important role in normal mammary gland development. The amplification is seen in up to 20% of breast cancers, which is significantly higher in lobular and with ER+/PR+ tumors. Coamplification of *MYC* and *CCND*1 occurs and is associated with aggressive phenotype. Coamplification with *FGFR1* has also been reported and is associated with worse outcome

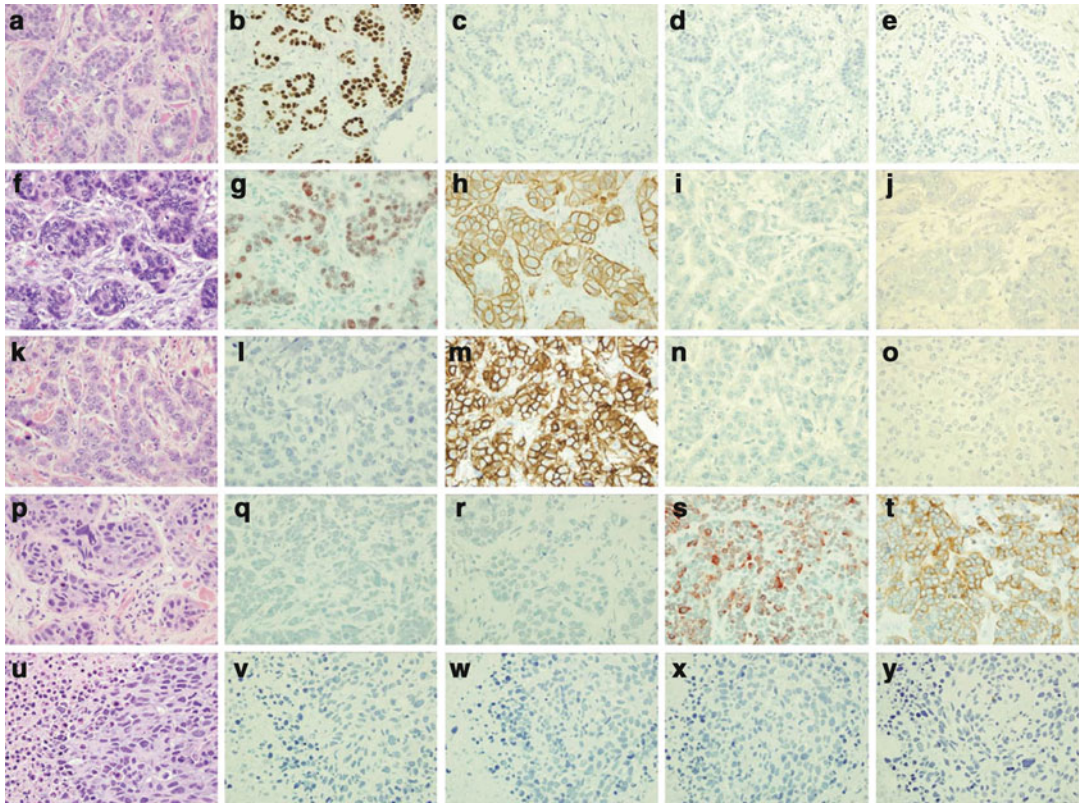


Fig. 6.11 Use of IHC in determination of molecular intrinsic subtypes. Representative cases for each molecular subtype. Hematoxylin and eosin and immunohistochemical stains of estrogen receptor, HER2, CK5/6, and epidermal growth factor receptor for luminal A (**a–e**), luminal B (**f–j**), HER2 (**k–o**), basal (**p–t**), and unclassified (**u–y**). (Adapted from Tang et al., 2009)

- 8p11.3 *FGFR1* on chromosome 8p11.3 is amplified in about 10% of breast cancers, and is associated with poor clinical outcome. Typically, it is associated with an ER+, PR+, and HER2− phenotype. In addition, *FGFR1* amplification is associated with resistance to endocrine therapy. *FGFR1* inhibitors have shown clinical response in patients with metastatic breast cancer, as an adjuvant to chemotherapy
- *MDM2 a*mplification has been reported in breast cancer and is associated with worse outcome in patients with node-negative disease
- Complex amplicons, as commonly observed with *HER2* on 17q22 (HER2) and *FGFR1* on8p11.3, typically involve a large number of adjacent genes that might also be important in the pathogenesis of breast cancer. For example, *TOP2A, RARA,* and *PPARB*. Coamplification with *TOP2A* is associated with responsiveness to anthracycline chemotherapy

Hereditary Breast Cancer BRCA1 and BRCA2

- Hereditary breast cancer (HBC) means that an alteration in a single major gene strongly contributes to the development of cancer or cancer-related conditions within the family. HBC was brought first to the medical literature by the surgeon Paul Broca, who accounted for

his wife pedigree in 1865 showing four generations of breast cancer and occurrences of cancer of the GI tract. In 1990, Hall et al. described a linkage specific site of breast cancer on chromosome 17q. *BRCA1* gene was later cloned. Subsequently, a second gene located in chromosome 13q was cloned, *BRCA2*. *BRCA1* and *BRCA2* are the major well characterized genes contributing to HBC, but others are known (but very rare), but it is likely that there are more yet to be discovered. In general, HBC is characterized by a significant earlier onset of breast cancer (average, 45, beginning at the age of 20), an excess of bilateralism, a greater frequency of multiple primary cancers (such as breast and ovary), and an autosomal dominant pattern of inheritance. In females, about 45% of HBC and 80% of hereditary breast and ovarian cancers are associated with *BRCA1* mutations. Most of the remaining HBCs are attributable to *BRCA2* mutations. The lifetime risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers is about 85%. The risk of ovarian cancer is 40–60% for *BRCA1* and 15% for *BRCA2*. It is estimated that about two-thirds of male breast cancer are linked to *BRCA2*, and one-third to *BRCA1* mutations. Overall, the prognosis of *BRCA1/2* mutated population appears to be similar to non-BRCA patients, although there is still controversy on this issue. For example, Ashkenazi Jews with *BRCA1/2* mutations appear to have relatively poor outcomes. Some new studies suggest that *BRCA1* patients may even have better survival than matched non-BRCA patients, and that *BRCA2* prognosis is worse

- *BRCA1*: 1,643 mutations have been described, of which 890 have been reported only once. For *BRCA2* approximately 1,856 mutations have been identified. BRCA shows two variants of penetrance, high (84% by 70 years of age) and low (32% by 70 years). Phenotypically, most *BRCA1* mutated tumors are basal-like breast cancers: highly proliferative, poorly differentiated, and genomically unstable. Most studies find *BRCA1* HBC to have a triple negative phenotype (ER−/PR−/HER2−). They are also associated with higher histological grade. A much higher prevalence of typical and atypical medullary carcinomas is also observed compared to sporadic breast cancers (35.3 vs. 3.4% for age matched controls and *BRCA1* mutated cancers). A lower prevalence of low grade tumors is seen in *BRCA1* mutated cancers compared to sporadic cancers, including ILC, tubulolobular, tubular, and invasive cribriform types. Indeed ILC commonly lack alterations at the *BRCA1* site. Aneuploidy is common among *BRCA1* mutated tumors. The frequency *of TP53* mutations is increased in *BRCA1* tumors compared to non-HBC and *BRCA2* tumors. Tamoxifen has been shown to be beneficial in reducing the risk of contralateral breast cancer in *BRCA1* patients, suggesting that they evolve from ER-positive precursors
- *BRCA2* mutated cancers have a more variable phenotypes than *BRCA1*, including a much higher proportion of luminal subtypes, and a much proportion of basal subtypes. Most studies show that the age of onset is older than in *BRCA1*. Some studies have shown higher prevalence of ILC associated with *BRCA2* than *BRCA1*. *BRCA2* also tend to show lesser aneuploidy and S phase. In *BRCA2*, ER/PR expression appears to be similar to non-BRCA cancer—a single study has even shown higher levels. Mutations of the *BRCA2* gene are also linked to other types of cancer, including pancreatic, prostate, and melanoma

Hereditary Breast Cancer Non-BRCA

- Non-BRCA HBC represents approximately 50% of cases in the general population. Overall, their clinical pathological features are statistically similar to sporadic breast cancer patients overall, including histological subtypes and grade, proliferation, p53 status, and intrinsic subtypes
- Germline mutations of *CDH1* (E-cadherin), which are very rare, confer a 40–70% lifetime risk of hereditary diffuse gastric carcinoma, and a 39–52% of ILC. E-cadherin is an adhe-

sion protein, which is lost in sporadic ILC through somatic mutations
- Li–Fraumeni syndrome: Lynch et al. described an extended kindred with a broad spectrum of cancers: sarcoma, breast cancer and brain tumors, lung and laryngeal cancers, leukemia, lymphoma, and adrenocortical carcinomas (SBLA syndrome). It is caused by a *TP53* germline mutation. The penetrance is variable with two age specific models: one in childhood and the second in adult life
- Cowden syndrome is a cancer associated genodermatosis, also referred as multiple hamartoma syndrome. It has an autosomal dominant pattern of inheritance, and is associated with distinctive mucocutaneous lesions and cancer of the breast, thyroid, and female genitourinary tract
- Germline mutations of the *PTEN* gene (also seen in Bannayan–Riley–Ruvalcaba syndrome). Cutaneous manifestations include trichilemmomas, which are pathognomonic. Also, multiple facial papules, acral and palmoplantar keratosis, skin tags and lipomas. Merkel cell carcinoma can occur. Thirty percent of women show breast carcinomas, and one-third shows bilateral disease. Patients with the mutation are candidates for prophylactic bilateral mastectomy

Familial Breast Cancer

- Familial breast cancer (FBC) is described as breast cancer within a family history of one or more first or second degree relatives affected. A patient with one or more first degree relatives with breast cancer in this category has a substantial excess lifetime risk of breast cancer when compared to patients in the general population. The relative risk increases from 1.80, 2.93, and 3.90 with one, two, and three first degree relatives compared to women without affected pedigree. FBC suggests a clustering of cancers that probably occurred by chance. In other words, there may be a combination of genetic and nongenetic (i.e., environmental) factors that contributed to the development of cancers within a family. In such instances, where an alteration in a single major gene is not likely or is not identified, individuals may still face elevated risks of cancer

Genome Sequencing of Breast Cancers

- Whole genome sequencing (WGS): The use of rapidly evolving techniques that combines whole genome, deep generation sequencing, and next generation sequencing have provided novel insights into the understanding of mutational analysis in breast cancer. Although these studies are in their infancy, it is already clear that essentially all breast cancers have an enormous number of mutations, far more than originally imagined—suggesting that developing widely successful targeted therapies will be extremely difficult. The seminal study by Sjoblom, based on outdated sequencing technology, found more than 100 distinct mutations in just 11 breast cancers. A more recent study Ding et al., using newer higher resolution technology, found an average of 50 somatic point mutations (including *JAK2, PTCH2, CSMD1, NRK, TP53*, *MAP3K8*), 28 large deletions, 6 inversions, and 7 translocations in a single case of basal-like breast cancer. One of the next major challenges in breast cancer research will be to determine which of the mutations are the "drivers" for developing breast cancer

Suggested Reading

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