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Preinvasive Breast Cancer

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Abstract

Preinvasive breast cancer accounts for approximately one-third of all newly diagnosed breast cancer cases in the United States and constitutes a spectrum of neoplastic lesions with varying degrees of differentiation and clinical behavior. High-throughput genetic, epigenetic, and gene-expression analyses have enhanced our understanding of the relationship of these early neoplastic lesions to normal breast tissue, and they strongly suggest that preinvasive breast cancer develops and evolves along two distinct molecular genetic and biological pathways that correlate with tumor grade. Although unique epigenetic and gene-expression changes are not observed in the tumor epithelial compartment during the transition from preinvasive to invasive disease, distinct molecular alterations are observed in the tumor-stromal and myoepithelial cells. This suggests that the stromal and myoepithelial microenvironment of preinvasive breast cancer actively participates in the transition from preinvasive to invasive disease. An improved understanding of the transition from preinvasive to invasive breast cancer will pave the way for novel preventative and therapeutic strategies.

Keywords

flat epithelial atypia; atypical ductal hyperplasia; ductal carcinoma in situ; genetics; gene-expression

Introduction

Breast cancer is a major health problem that affects the lives of millions of women worldwide each year. In 2008 in the United States alone, approximately 180,000 women were diagnosed with invasive breast carcinoma, and there were 67,000 new cases of preinvasive breast cancer (1). Over the past several decades, the incidence of breast cancer has risen, while the death rate from breast cancer has been steadily decreased. These seemingly contradictory observations may be explained in part by increased mammographic screening and improved diagnostic recognition of the earliest curable preinvasive stages of breast cancer (2).

Approximately 80% of all diagnosed preinvasive and invasive breast cancers in the United States are of the ductal subtype (3). This specific subtype is by far the most comprehensively

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studied group of breast tumors at the clinical, histopathological, immunohistochemical, and molecular levels. Thus, with the exception of a brief histomorphological and cytogenetic review of atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS), the two preinvasive lesions of the lobular subtype, this review focuses predominantly on the preinvasive lesions of the ductal subtype with special emphasis on the molecular pathology of these ductal lesions as they relate to breast cancer evolution and progression. In addition, this review covers recent advances in our understanding of the tumor microenvironment of preinvasive breast cancer and concludes with a brief discussion of the emerging area of molecular prognostic biomarkers of preinvasive breast cancer.

THE ORIGIN OF HUMAN BREAST CANCER

Currently, there are two prevailing models of breast cancer tumorigenesis and evolution: the stochastic model and the cancer stem cell model of carcinogenesis (4, 5). The stochastic model (also known as the clonal evolution model) postulates that transformation originates from random mutations in any breast epithelial cell (stem cell, progenitor, or differentiated cell) and that the neoplastic process further evolves through accumulation of random genetic events that drive uncontrolled proliferation and resistance to apoptosis (**Figure 1a**) (6-8). Over time these, neoplastic cells in resultant tumors undergo additional genetic and epigenetic changes and coevolve with their microenvironment, leading to cellular heterogeneity within a tumor (9, 10).

According to the cancer stem cell hypothesis, all tissues are derived from organ-specific stem cells that are defined by their capacity to undergo self-renewal as well as to differentiate into the cell types that constitute each organ (4). In normal adult breast tissue, stem cells are relatively quiescent, long-lived cells that are defined by their ability to self-renew and to generate progenitors that differentiate into the different functional [estrogen receptor (ER)- positive and ER- negative luminal epithelial and myoepithelial] cells of the breast. The cancer stem cell hypothesis has separate but related components that include the following. 1. The cellular origin of breast cancer is the same as that of tissue stem cells or progenitor cells, and akin to what has been observed in human hematological malignancies (11, 12), genetic transforming events occurring at different development points in the breast stem cell continuum account for breast cancer phenotypic heterogeneity in the form of the major subtypes of breast cancer (**Figure 1>b**) (13). 2. Breast cancers are driven by cellular components that display stem cell-like properties of self-renewal (4).

Compelling and reproducible data support both the stochastic model and cancer stem cell model, and it is likely that the origin of the earliest preinvasive stages of breast cancer results from elements that can be encompassed by both theories. Although the origin of human breast cancer is an area of intense research, this review focuses on molecular alterations that occur subsequent to the breast cancer- initiating events.

PREINVASIVE STAGES OF BREAST CANCER: A BRIEF HISTOPATHOLOGICAL REVIEW

This section begins with a brief histomorphological review of normal breast tissue and of the preinvasive stages of breast cancer. This summary provides for a more complete conceptual framework from which to build a contemporary molecular-based model of progression. The breast is composed of a progressive branching system of ducts that originate at the nipple and end in one of many terminal ductal lobular units (TDLUs), which are the smallest functional units of the breast (**Figure 2**). A TDLU is composed of a single terminal duct (TD) and multiple end ductules (or acini) invested in a specialized stromal compartment. The ductal system of the breast and the TDLU is lined by two cell layers: (a) the inner luminal epithelial layer that consists of low columnar and cuboidal cells of the TD and acini, respectively, and (b) the outer myoepithelial layer that abuts the basement membrane. By

definition, the preinvasive epithelial lesions of the breast are characterized by a neoplastic epithelial cell proliferation that is confined within the ductal-lobular network of the breast without invasion through the basement membrane into the surrounding breast stromal compartment (14).

The preinvasive stages of breast cancer, similar to the invasive stages of breast cancer, fall into two general histologic categories: the lobular and ductal subtypes. The historic view that the lobular subtype arises from lobules and that the ductal subtype arises from ducts is misleading; the seminal studies by Wellings and colleagues (15, 16) have demonstrated that most breast cancers (both ductal and lobular types) arise in the same microanatomical site, the TDLU. The distinction of the lobular subtype from the ductal subtype is based upon differences in cell morphology. More specifically, the lobular subtype consists of small, nonpolarized cells that resemble the low-cuboidal luminal cells of normal breast acini, whereas the ductal subtype consists of moderate to large, frequently polarized cells that resemble the low columnar cells of the normal breast ducts. The preinvasive stages of lobular breast cancer include ALH and LCIS, whereas the equivalent lesions of the ductal subtype include flat epithelial atypia (FEA), atypical ductal hyperplasia (ADH), and ductal carcinoma in situ (DCIS).

ALH and LCIS, two types of preinvasive lesion grouped under the term lobular neoplasia, are considered as nonobligate precursors of invasive lobular carcinoma (ILC) (17-19). The histomorphological differentiation of ALH from LCIS is based on the extent of proliferation and the distension of the TDLU (**Figure 3**). In ALH, a TDLU is partly colonized by a monomorphic population of small, round, nonpolarized, loosely cohesive cells with a high nuclear-cytoplasmic ratio. In general, the proliferation of ALH is not widespread, and does not usually obliterate the acinar lumina. So-called classic LCIS consists of a population of cells with identical cytomorphological characteristics of ALH, but in which the colonized acini of the TDLU are filled and distended with the neoplastic cells (20). A pleomorphic variant of LCIS has been described and occurs less frequent than the classic type (21). Histomorphologically, pleomorphic LCIS consists of medium- to large-sized pleomorphic cells with or without associated necrosis (**Figure 3**) (21). The loss of expression of membrane E-cadherin is a hallmark feature of ALH and LCIS (19, 22). However, this feature is not pathognomonic of these lesions, as ductal carcinomas can also demonstrate loss of E-cadherin (23).

FEA, ADH and DCIS are considered the nonobligate precursors of invasive ductal carcinoma (IDC). FEA-- also referred to in the literature as atypical cystic lobule, columnar cell change or hyperplasia with atypia, columnar alteration with prominent apical snouts and secretions with atypia, and hyperplastic enlarged lobular units (24, 25)-- is characterized by a minimal proliferation and replacement of native luminal cells of the TDLU by one to several layers of monomorphic cuboidal or columnar epithelial cells with low-grade cytological atypia (**Figure 3**). Notably, the cells of FEA do not pile up to fill the TD and acini [as they do in usual ductal hyperplasia (UDH)], but rather grow as a single or minimally pseudostratified layer that enlarges and distends the TDLU (24). FEA differs from the next stage of progression, ADH, in that the latter is characterized by both low-grade cytological atypia and architectural atypia in the form of micropapillae, trabecular bars, Roman arches, cribriform spaces and solid growth. Historically, the pathological distinction between ADH and DCIS is considered by some to be based upon the degree of architectural atypia and the size and the extent of epithelial proliferation (26, 27). Through use of conventional histomorphological parameters that include cytomorphological and architectural features, DCIS is further subclassified into low-, intermediate-, and high-grade categories. Lastly, the histomorphological feature that distinguishes IDC from DCIS is the

disappearance of the outer organized myoepithelial layer along with growth of the neoplastic cells beyond the basement membrane and into the surrounding stroma (14).

HISTOPATHOLOGICAL-BASED MODELS OF HUMAN DUCTAL BREAST CANCER PROGRESSION

Histopathological and epidemiological observations over the past century have resulted in two well-recognized linear models of breast cancer progression (**Figure 4**). The classical model, derived from careful histomorphological observations first described more than 100 years ago (28) and further refined by Wellings and colleagues (15, 16), postulates that an initiating event within an epithelial cell of the TDLU gives rise to FEA. A proliferative growth advantage within FEA is postulated to spawn ADH, upon which additional molecular alterations give rise to the last preinvasive stage of breast cancer progression, DCIS. Finally, progressive molecular events give rise to the malignant (or potentially lethal) stages of IDC and metastatic carcinoma (29).

It was not until the influential studies by Page and colleagues (30, 31) that the alternative model became popular. The alternative linear model of breast cancer progression is identical to that of the classical model with the exception of the introduction of UDH, rather than FEA, as the direct precursor to ADH (**Figure 4**). Historically, the role of UDH as the direct precursor lesions to ADH was supported by the epidemiologically based studies demonstrating that women with a benign breast biopsy showing UDH have a mildly elevated risk of breast cancer (~1.5 to 2.0 times that of the reference population), and that women with ADH have a substantial increased risk for developing breast cancer (~3.5 to 5.3 times that of the reference population) (31). Although this epidemiological evidence supported UDH as the precursor to ADH, recent immunohistological and molecular pathological evidence, as described below, strongly suggests that UDH is not a precursor to ADH and that the alternative linear model of progression is probably invalid.

MOLECULAR CLASSIFICATION OF INVASIVE DUCTAL BREAST CANCER

Major progress in the molecular classification of invasive breast cancer has been achieved through the combined use of immunohistochemistry, gene-expression and genomic-based technologies. The molecular classification of invasive breast cancer serves as an important reference point for the following discussion of preinvasive breast cancer.

Genomic Analysis of Invasive Ductal Carcinoma

Tumor heterogeneity is a well-recognized and clinically relevant, but poorly understood, property of invasive breast cancers. Traditionally, classification of IDCs into clinically meaningful groups has been performed through histological grading systems (see sidebar, Histopathological Grading) (32, 33). Several groups studying the relationship of genetic alterations with histological tumor grade have made important contributions to our understanding of breast cancer classification and breast cancer evolution (34-36). These groups demonstrated that specific tumor grades exhibit distinct genomic differences in which fewer chromosomal aberrations occur in low grade tumors as compared with high grade tumors. These general quantitative genetic differences are also associated with distinct qualitative differences. Grade I tumors (grade I tumors are well differentiated, whereas grade III tumors are poorly differentiated) display frequent recurrent chromosomal loss of 16q and gains of 1q, 16p and 8q, whereas high-grade tumors display frequent high-level amplifications of 17q12 and 11q13; losses of 8p, 11q, 13q, 1p, and 18q; and gains of 1q, 8q, 17q, 20q and 16p (**Figure 5**) (35, 36). Intermediate-grade tumors share genomic alterations common to either low-grade or high-grade carcinomas, suggesting that this population of tumors consists of mixture of each type (37). The most significant finding as it relates to

breast cancer classification and breast cancer evolution is the frequent versus infrequent loss of 16q in low-grade and high-grade IDCs, respectively (35, 36). This distinct pattern of chromosomal loss has led some investigators to speculate that the majority of grade I carcinomas do not evolve to grade III tumors through dedifferentiation, as such an evolutionary scheme would necessitate the recovery of lost genetic material (29, 35). Taken together, all of these observations support a hypothesis of early divergence between the two main subtypes of IDCs (**Figure 5**) and support a modified model of breast cancer progression.

Gene-Expression Analysis of Invasive Ductal Carcinoma

Over the past seven years, multiple independent research groups have conducted genome-wide expression profiling studies in an effort to identify novel diagnostic, prognostic and predictive classification schemes as a means to guide clinical decision-making (38-49). In an influential study, Perou et al. (50), using microarray gene-expression profiling, revealed a remarkably consistent molecular classification scheme in which breast cancers can be classified into four distinct intrinsic categories that include (a) two subtypes of ER-negative tumors, the basal-like and ERBB2 subtypes, and (b) two ER-positive tumors, the luminal A and luminal B subtypes. The basal-like tumors typically lack expression of ER, progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) and express cytokeratins 5/6 (basal cytokeratins) and epidermal growth factor receptor (EGFR) (50, 51). The *ERBB2* tumors are characterized by expression of HER2 and by lack of expression of ER and PR (50, 51). Luminal A tumors are characterized by ER and PR expression with the lack of HER2 overexpression, whereas luminal B tumors typically express ER and overexpress HER2 with or without PR expression (50, 51). Subsequent follow-up studies demonstrated that beyond gene-expression differences these four specific molecular subtypes were associated with distinct clinical outcomes (39, 40).

Since these initial observations, multiple seemingly novel breast cancer gene-expression signatures have been identified (41, 44, 45, 52, 53). Such signatures consist of fairly unique gene sets with very little to no overlap, yet they all have a similar ability to predict breast cancer outcome. Important issues are whether these different gene-expression signatures share a unifying biological principle that translates into a common clinical overlap in their prognostic information and whether combining several of these signatures would provide more accurate risk assessment. To address this issue, Fan et al. (54), compared the prognostic performance of four different breast cancer gene-expression signatures and demonstrated that all four signatures were highly concordant in classifying patients into low- and high-risk groups. A combination of the four signatures did not significantly improve upon the prognostic predictive accuracy of each signature alone, suggesting that the prognostic information captured by these signatures is largely overlapping and that it probably reflects a common biological principle (54). All four signatures were significantly correlated with tumor grade, suggesting that the unifying biological principle captured by these signatures may be rooted in this well-established pathological parameter (54). Subsequent comparative gene-expression studies indicate that these seemingly disparate breast cancer gene-expression signatures share common molecular pathways centered on cell-cycle regulation and cell proliferation (55-58). Thus, the common biological principle of cell proliferation, which is the most important component of histological grading (59-62), is the main driving force behind the prognostic power of these biomarkers.

Although the histopathological grading system recognizes three distinct clinico-pathological subgroups, the above-described genomic studies suggest that a two-category grading system may be more biologically and clinically relevant. Using a so called bottom-up approach, Sotiriou and colleagues (38) and Ma and colleagues (63) identified both complex [97-gene

genomic grade index, (GGI)] and a simple [five-gene molecular grades index, (MGI)] tumor grade gene-expression signatures, which are differentially expressed between low- and high-grade tumors. Consistent with the view that molecular grading is superior to subjective histomorphological grading, these signatures successfully classified intermediate-grade tumors into two groups with similar clinical outcomes: low grade-like and high grade-like tumors (**Figure 5**) (38). Given the aforementioned data which suggest that most gene-expression signatures reflect tumor grade and proliferation, Sotiriou and colleagues (58) recently performed an unbiased comparison of 70- and 76- gene-expression signatures to the GGI in the TRANSBIG clinical trial series and demonstrated that GGI, a pure tumor grade-based gene-expression biomarker, exhibited prognostic performance highly similar to those of both the 70-gene and the 76-gene-expression signatures. Another study by Sotiriou's group (64) demonstrated that GGI and the 21-gene signature had similar prognostic performance, and our group (63) recently demonstrated that the simple five-gene MGI biomarker possesses prognostic equivalence to the more complex 97-gene GGI assay. Therefore, despite investigators' differing approaches to the development of various gene-expression signatures, most if not all gene-expression signatures are an objective surrogate measure of tumor grade and proliferation and are highly similar in terms of prognostic performance. In summary, the molecular interrogation of invasive breast cancer strongly suggests that tumor grade, more than any other clinicopathological parameter, strongly reflects the extent and type of underlying genetic and gene-expression alterations, and that these alterations represent two distinct evolutionary, biological and clinicopathological pathways (**Figure 5**).

MOLECULAR CLASSIFICATION OF THE PREINVASIVE STAGES OF DUCTAL BREAST CANCER PROGRESSION

Until recently, a significant impediment to a better understanding of breast cancer progression was our inability to readily and accurately interrogate and assess the molecular events associated with the early preinvasive stages as they relate to invasive carcinoma. However, over the past decade the successful combination of highly specific tissue-microdissection technologies with advanced high-throughput genomic, gene-expression and proteomic technologies has reshaped our view of the preinvasive stages of breast cancer progression. As described below, significant genomic and gene-expression parallels exist between the preinvasive and invasive stages of breast cancer. The molecular characterization of DCIS is described first, as this well-characterized lesion serves as the logical reference point for ADH and FEA.

GENOMIC ANALYSIS OF THE PREINVASIVE STAGES OF DUCTAL BREAST CANCER

Similar to what has been observed for IDC, DCIS represents a spectrum of neoplastic diseases. Some of these behave in an indolent manner, whereas others behave in a more aggressive manner (see sidebar, Natural History of Preinvasive Breast Cancer). Multiple morphologically based schemes have been proposed as a means to better to classify DCIS biologically and clinically (65-68). Several comparative studies have revealed that DCIS is a genetically advanced lesion and that different morphological subtypes of DCIS mirror distinct genomic alterations characterized by loss of 16q in low-grade DCIS and amplification of 17q12 in high-grade DCIS (69, 70). More specifically, Buerger et al. (69) performed a comparative genomic hybridization (CGH)-based analysis of DCIS and invasive carcinoma and revealed that losses of 16q were seen almost exclusively in low- and intermediate-grade DCIS, whereas a higher frequency of 1q gain and 11q loss was observed in intermediate-grade DCIS. High-grade DCIS, however, demonstrated complex genomic alterations characterized by loss of 8p, 11q, 13q and 14q, by gains of 1q, 5p, 8q and 17q, and high-level amplifications of 17q12 and 11q13 (69). Analysis of CGH data generated from synchronous and metachronous IDC and DCIS lesions revealed a near-identical pattern of

genetic change supporting the direct precursor relationship between DCIS and IDC (36, 37, 69). In a recent study utilizing CGH in conjunction with serial analysis of gene expression (SAGE), Yao et al. (71) demonstrated an overall trend toward an increase in the number and amplitude of gains and losses during breast cancer progression, which supports the concept that the early neoplastic stage of DCIS is a direct precursor to IDC. Taken together, these data provide evidence that DCIS is a direct precursor to IDC and, furthermore, that distinct genetic pathways captured by the clinicopathological parameter of tumor grade exist within the morphologically diverse spectrum of DCIS.

Histomorphologically, ADH shares some but not all of the architectural and cytological features of low-grade DCIS, and the diagnostic criteria separating the two lesions are predominantly quantitative, rather than qualitative, in nature (26, 27). These pathological features, along with clinical and epidemiological data, support the role of ADH as the precursor to low-grade DCIS. Additional evidence in support of this hypothesis is provided by the results of multiple loss of heterozygosity (LOH)-based and CGH-based studies. Several small studies revealed losses of chromosomal regions 16q, and 17p in ADH (72-74) and showed that the frequency of chromosomal losses in these regions is similar to that observed in DCIS and IDC. A comprehensive study by O'Connell and colleagues (75) who analyzed LOH in 399 preinvasive breast lesions, revealed LOH in at least 1 of 15 loci studied in 42% and 44% of ADH lesions from noncancerous and cancerous breasts, respectively. Furthermore, this group identified chromosome 16q as an LOH hot spot in ADH and showed that this hot spot was more frequently shared with low-grade (noncomedo) DCIS than high-grade DCIS (75). Given the considerable difficulties in histomorphologically discriminating between ADH and low-grade DCIS, it is not surprising that these two preinvasive lesions share a common chromosomal abnormality. The morphological overlap that is reflected at the molecular level in these two lesions has led some researchers to question the validity of classifying ADH and low-grade DCIS as distinct and separate pathological entities (76). Currently, ADH is the accepted precursor lesion for low-grade DCIS. However, the precursor lesion for the majority of high-grade DCIS cases (those with 17q12 amplification; see **Figure 6**) remains elusive.

As mentioned above, some epidemiological and clinical data support the alternative model of breast cancer progression in which UDH is postulated to be the precursor lesion of ADH. However, multiple lines of evidence suggest otherwise and point to FEA, rather than UDH, as the precursor to ADH and low-grade DCIS. First, morphologically speaking, the cells that constitute FEA are characterized by variable degrees of low-grade cytological atypia that forms a continuum from normal lobules to ductal carcinoma in situ, whereas UDH consists of a population of immature mammary epithelial cells that can undergo divergent differentiation as either glandular epithelial cells or myoepithelial cells (77). Second, immunohistochemical profiles of FEA are nearly identical to those of ADH and low-grade DCIS with FEA demonstrating diffuse positivity for ER, PR and cytokeratin 19 (CK19) (70, 78), variable but increased staining for cyclin D1 (78), and nearly uniform negativity for HER2 (70, 79) and CK5/6 (70). Immunohistochemical profiles of UDH, however, show a mixed population of cells with a variable proportion of CK5/6-positive myoepithelial and basal cells and ER+, PR+, CK8/18/19-positive glandular (luminal) epithelial cells (80, 81). Third, at the molecular level, FEA's genetic profile overlaps with those of synchronous low-grade DCIS and low-grade invasive carcinoma (82). More specifically, Moinfar and colleagues (82) noted a particularly high rate of LOH at chromosome 16q, a locus commonly altered in low-grade DCIS. A more recent comprehensive CGH-based study by Simpson et al. (70) extended these observations by demonstrating that FEA exhibits recurrent chromosomal copy number gains and losses, (gains on 15p, 16p and 19; losses on 16q, 17p and X), and that these recurrent genetic alterations have significant overlap with those observed in both ADH and low-grade DCIS. However, although LOH is observed in

UDH, the pattern of LOH is notably different from that associated with ADH and DCIS (75, 83-88). More specifically, only rare and fairly randomly distributed chromosomal changes, or no changes at all, occur in UDH (a pattern similar to that observed in phenotypically normal breast tissue and nonproliferative fibrocystic change), whereas recurrent, nonrandomly distributed chromosomal changes (in particular 16q loss) occur more frequently in ADH and DCIS (69, 75, 85, 86, 88-90). Taken together, these observations support the role of FEA as the precursor to ADH. They also question the role of UDH as a precursor to ADH and, thus, the validity of the alternative model of breast cancer progression.

GENE-EXPRESSION ANALYSIS OF THE PREINVASIVE STAGES OF DUCTAL BREAST CANCER

Over the past several years, there has been considerable research interest in understanding the gene-expression changes that occur during the early preinvasive stages of breast cancer (91-97). It is particularly noteworthy, that these studies focused primarily on the gene-expression changes within the neoplastic epithelial cells that constitute ADH and DCIS; comprehensive gene-expression profiling of FEA has not been reported to date. One of the earliest and most comprehensive studies is that done by Ma and colleagues (94), in which both patient-matched phenotypically normal breast epithelium (N) from the TDLU and epithelium constituting ADH, DCIS and IDC were microdissected and hybridized to a complementary DNA (cDNA) microarray containing 12,000 genes. Not unexpectedly, comparative gene-expression profile analysis of patient-matched N versus ADH, N versus DCIS, and N versus IDC revealed that the most pronounced transcriptional changes occur at the N-to-ADH transition and that such transcriptional alterations are maintained throughout the later stages (DCIS and IDC) of progression (94). Unexpectedly however, on a global level no consistent major transcriptional changes between the preinvasive and invasive stages were identified preinvasive. This finding has been reported in two additional studies (93, 95). Taken together, these data support the idea that the different stages of breast cancer progression are evolutionary products of the same clonal origin and suggest that gene-expression patterns expressed in the preinvasive stages (ADH and DCIS) of disease may, in fact, reflect the progressive potential of the pathological lesion. The concept that gene-expression of an early-stage breast cancer may predict future clinical behavior is supported in the literature by the repeated observations that gene-expression patterns in early stage invasive breast cancer predict the risk of distant metastases (39-41, 43, 44, 98-102).

Despite their use of different gene-expression microarray platforms (cDNA arrays, oligonucleotide arrays, and SAGE), several studies have demonstrated that the transition from the preinvasive stage of DCIS to invasive carcinoma is associated with quantitative, rather than qualitative, differences in gene-expression (94-96). More specifically, these studies have identified subsets of genes that are consistently overexpressed in IDC relative to patient-matched DCIS. Similarly, analyses of breast cancer development in several transgenic mouse models also demonstrate that the transition from a preinvasive to an invasive stage of progression is associated with quantitative, rather than qualitative differences in gene-expression (103, 104). This quantitative relationship is most prominent in high-grade (poorly differentiated/grade III) samples, revealing an intriguing link between tumor grade and tumor-stage progression (94). All of these observations suggest that breast cancer progression may be more complex than envisioned by the current linear theory of activation and inactivation of oncogenes and tumor suppressor genes, respectively, and that it may be dependent upon such contingencies as quantitative levels and timing of gene-expression.

Although the study by Ma and colleagues (94) did not identify gene-expression differences that are specific to the distinct preinvasive and invasive stages of breast cancer, unique gene-

expression alterations have been associated with different tumors grades (38, 94, 105, 106). Notably, similar to what has been observed with invasive breast cancer, distinct gene-expression signatures are present in low- and high- grade DCIS lesions (94). Low-grade DCIS and ADH lesions share a common gene-expression signature populated with genes associated with the ER phenotype, whereas high grade DCIS lesions possess a gene-expression signature populated with genes associated with increased mitotic-activity and cell-cycle processes (94). Thus, taken together these gene-expression data further support the concept that low-grade and high-grade preinvasive neoplasms arise from two distinct evolutionary pathways (Figure 6) and that intermediate-grade DCIS represents a mixture of low- and high- grade neoplasms (38, 94, 106).

MOLECULAR ANALYSIS OF INVASIVE AND PREINVASIVE LOBULAR BREAST CANCER

As compared with those of ductal breast cancer, molecular analyses of the invasive and preinvasive stages of lobular breast cancer are somewhat limited. Conventional cytogenetic- and array CGH-based studies demonstrate some differences in the patterns of genetic alteration in ILC as compared with those of IDC (36, 107-110). As the majority of ILCs consist of low-nuclear-grade malignant cells (i.e., classic ILCs), it is not surprising that ILCs share a recurrent loss of 16q with low-grade IDCs (107-110). This finding supports the hypothesis that ILCs and low-grade IDCs may share a common pathway of tumorigenesis (29). In a minority of ILCs, the tumor consists of high-nuclear-grade malignant cells (i.e., pleomorphic ILC). Interestingly, these tumors share genetic changes with both classic ILCs and high-grade IDCs in the form of (a) 16q loss and 1q gain and (b) amplification of 17q12, respectively (111, 112). However, a recent comparative analysis of array CGH data demonstrated that the overall molecular features of pleomorphic ILC are more closely related to those observed in ILC than those seen in IDC, suggesting that pleomorphic ILCs share a common evolutionary association with classic ILC along the low-grade, ER-positive pathway of neoplastic development and that they do not represent high-grade IDCs (112). Relatively few studies have focused on the genomic analysis of the preinvasive stages of lobular breast cancer (113-116). Lu and colleagues (114) performed a CGH-based analysis of ALH and LCIS; they demonstrated a similar pattern of chromosomal imbalance with the frequent chromosomal loss of material from 16p, 16q, 17p and 22q. Notably, no statistically significant differences between ALH and LCIS were identified, providing evidence that these two lesions are not only related but are also at a similar stage of progression (114). More recently, Mastracci et al. (115) further refined the molecular signature of ALH and LCIS by identifying novel loss of 7p11 and 22q11 and gain of 2p11 in ALH, and novel loss of 19q13 and gain of 20q13 in LCIS. Most importantly these studies, as well as that by Morandi and co-workers (116), demonstrated the common loss of 16q in ALH and LCIS with ILC, supporting an evolutionary link among the three types of lesions. Gene-expression profiling of classic ILC and LCIS reveals a gene-expression signature that overlaps significantly with that observed in low grade IDCs (117). Thus, this gene-expression data, in conjunction with the CGH data, support a common evolutionary –16q, low-grade gene-expression pathway (**Figure 6**) that encompasses (a) ILC and its precursor lesions (ALH and LCIS), and (b) low-grade IDC and its precursor lesions (FEA, ADH and low-grade DCIS).

A CONTEMPORARY EPITHELIAL-CENTRIC MODEL OF BREAST CANCER PROGRESSION

The traditional linear model of carcinogenesis put forth by Wellings and colleagues (15, 16) is one of progression from normal breast epithelium to FEA to DCIS to IDC. However, both mathematical (118, 119) and molecular biological analyses question the validity of this morphological-based model. As outlined above, comparative genomic and gene-expression analyses of the different stages of breast cancer suggest that breast carcinogenesis evolves along one of two distinct branched pathways of progression defined by tumor grade and loss of chromosome 16q (the two pathway model; **see Figure 5**). Thus, these molecular studies

account for phenotypic heterogeneity among different tumors. However, such studies do not address the phenomenon of heterogeneity, which is defined by variation in the degree of tumor cell differentiation (i.e., tumor grade) and is frequently encountered in both the in situ and the invasive stages of ductal breast cancer progression. Recently, breast cancer intrastage heterogeneity was specifically addressed by Allred and colleagues (25), who performed immunohistochemical, morphological (nuclear grade) and gene-expression comparative analysis of 200 cases of pure DCIS. The authors showed that multiple histologic grades, biomarker phenotypes, and intrinsic subtypes often coexist within the same DCIS lesion (25). Importantly, these data support further refinement of our model of breast cancer progression to include intrastage heterogeneity in which a subset (~9%) of high-grade DCIS can evolve from their low-grade counterparts (25). Although the observations by Allred and colleagues appear incongruent with a simple two-pathway model of progression, recent data by Natrajan et al. (120) may provide a unifying corollary to the two-pathway model. Specifically, Natrajan and colleagues demonstrated increased frequency of 16q loss in high-grade tumors of the luminal type as compared with the other intrinsic types, suggesting that the evolutionary progression of a low-grade tumor to a high-grade tumor may preferentially occur in breast cancers of the luminal (low-grade, ER positive) phenotype (120). Thus, in light of the recent data by Allred et al. and Natrajan et al., our contemporary two-pathway model of progression must be further refined to account for intrastage heterogeneity through the process of intrastage tumor-grade evolution within the –16q low-grade gene-expression molecular pathway (**Figure 6**).

MOLECULAR ANALYSIS OF THE NON-EPITHELIAL CELLS OF THE TUMOR MICROENVIRONMENT

Most breast cancer researchers have traditionally employed a reductionist approach to exploring tumors by focusing on the cancer cells and the genes within them. Although this tumor epithelial-centric approach is conceptually satisfying, it may be too simple. The neoplastic epithelial cells of breast cancer coexist with several types of nonneoplastic cells that together create the tumor microenvironment. Myoepithelial and inflammatory cells constitute the intraluminal tumor microenvironment of the preinvasive stages of breast cancer, whereas fibroblasts, myofibroblasts, inflammatory cells, and endothelial cells constitute the stromal microenvironment of invasive breast cancer. Although these nonneoplastic cells have been generally considered a silent or reactive bystanders within breast tumors, several lines of evidence suggest that active bidirectional signaling between malignant breast epithelial cells and the nonneoplastic cells of the tumor micro-milieu plays a critical role in breast tumorigenesis and progression. First, both in vitro- and in vivo-based assays of breast tumorigenesis have demonstrated that experimental manipulation of the stromal microenvironment has profound effects on tumor cell growth, invasion and metastasis (121-124). Second, normal myoepithelial cells have been demonstrated to exert an autocrine- and paracrine-mediated pleiotropic suppressive effect on breast cancer progression (125, 126). Third, multiple epithelial-centric, comprehensive gene-expression profiling studies have failed to identify breast cancer stage-specific alterations. These findings strongly suggest that tumor-stromal microenvironmental alterations, rather than tumor-intrinsic alterations, may orchestrate tumor-stage progression (94, 95). As a result of these observations, there has been an intense resurgence of interest in the tumor microenvironment of both invasive and preinvasive breast cancer.

To better understand the potential role of the tumor microenvironment in human breast tumorigenesis, Allinen et al. (127) performed a comprehensive genomic and gene-expression analysis of ex vivo-isolated neoplastic epithelium as well as the nonneoplastic cells that constitute the tumor microenvironment of preinvasive and invasive breast cancer.. Allinen and colleagues demonstrated consistent and dramatic gene-expression changes in

the nonneoplastic myofibroblastic cells of the tumor-stromal microenvironment and in the nonneoplastic myoepithelial cells of the intraluminal tumor microenvironment of DCIS, as compared with myofibroblastic and myoepithelial cells of normal breast tissue. Closer analysis of these changes demonstrated that DCIS-associated myoepithelial cells, when compared with normal myoepithelial cells, show upregulation of genes encoding proteases such as *cathepsins F, K and L*, and *metalloproteinase 2 (MMP2)*, as well as chemokines such as *CXCL12/SDF-1* and *CXCL14*, both of which have been implicated as regulators of cell growth, migration, and invasion (128-131). Recent findings by Orimo et al. (132) strongly suggest that human breast cancer-associated stromal cell secretion of CXCL12/SDF-1 promotes neoplastic epithelial growth in invasive carcinomas by direct paracrine stimulation. Consistent with these findings, Allinen et al. (127) observed an increased frequency of mitotic activity in malignant epithelial cells adjacent to the myoepithelial cells as compared with other regions of DCIS. This finding suggests that heterotypic paracrine signaling involving chemokines occurs in the preinvasive stage of breast cancer.

To determine whether the dramatic gene-expression changes in the neoplastic epithelial cells, as well as in the myoepithelial and myofibroblastic cells, could be due to underlying genetic alterations, Allinen et al. (127) further performed comprehensive array-CGH-based analysis of such ex vivo-procured cells in normal breast tissue as well as DCIS and invasive breast cancer samples. As expected, they detected no genetic alterations in epithelial or myoepithelial cells isolated from normal breast tissue located adjacent to these tumors. Furthermore, in agreement with previous studies (69, 133), the authors observed significant genetic alterations in the neoplastic epithelium of both preinvasive and invasive breast cancers. However, in contrast to previous observations (134-136), no genomic changes were detected in tumor-associated myofibroblastic or myoepithelial cells. To further demonstrate the lack of genomic alterations in the myoepithelial and myofibroblastic cells, the authors performed a comprehensive genome-wide single nucleotide polymorphism (SNP) array analysis, as well as direct sequencing of SNPs, and found no conclusive evidence for genetic alterations in these stromal cells. A lack of clonal genetic change in breast cancer associated stromal cells has been further confirmed by Qui and colleagues (137). These data as they specifically relate to tumor-associated stromal cells are discrepant with previous findings. However, as pointed out by Polyak and colleagues, this discrepancy probably due to technical limitations associated with the previous published approaches (9, 127). Taken together these data demonstrate that although nonneoplastic tumor-associated myoepithelial cells of DCIS and myofibroblastic cells of invasive carcinoma are phenotypically different (i.e., gene-expression) from their normal counterparts, genomic changes detected by comprehensive array CGH and SNP analyses are limited to the neoplastic epithelial cells (127).

Significant gene-expression changes in nonneoplastic stromal and myoepithelial cells, without obvious genetic alterations, suggest that epigenetic modifications may be responsible for alterations in these cells. Utilizing methylation-specific digital karyotyping, Hu et al. (138) explored the possibility that epigenetic alterations underlie the relatively stable gene-expression phenotype observed in nonneoplastic stromal cells of preinvasive and invasive breast cancer samples. First, as expected, distinct epigenetic alterations between normal breast epithelium and tumor epithelium were observed. Second, epigenetic changes between normal stroma and tumor stroma were also seen. Third, and most notably, similar to stromal epigenetic differences observed between normal breast and invasive breast cancer tissues, distinct recurrent epigenetic alterations were observed in DCIS-associated myoepithelial cells as compared with their normal counterparts. The latter finding strongly suggests that epigenetic changes in nonneoplastic myoepithelial cells play an important role in the establishment and maintenance of the abnormal tumor microenvironment in the preinvasive stage of breast cancer (138).

More recently, Ma et al. (93) used laser capture microdissection and oligonucleotide microarrays to conduct an in vivo-based comparative global gene-expression analysis in the epithelial and stromal compartments during breast cancer progression from normal to preinvasive to IDC. Again, consistent with previous observations, major epithelial gene-expression changes were shown to occur at the normal-to-DCIS transition, whereas no major epithelial gene-expression differences were identified at the transition from DCIS to IDC. In the stromal compartment, as in the epithelial compartment, thousands of gene-expression changes occurred at the transition from normal to DCIS. The top differentially expressed genes between DCIS-associated stroma and normal breast stroma include several signaling pathways previously implicated in human breast cancer. Two antagonists of WNT receptor signaling *WIF1* and *SFRP1*, were consistently downregulated in the DCIS stroma, while *GREM1* and *INHBA*, two transforming growth factor beta (TGF- β) family members, were strongly induced in DCIS-associated stroma. These observations confirm previous findings (139, 140) and strongly suggest that decreased expression of *WIF1* and *SFRP1*, and increased expression of *GREM1* and *INHBA* in the tumor-stromal microenvironment may play an important role in the early stages of breast cancer progression. However, unlike the epithelial compartment that demonstrates no or rare gene-expression changes at the transition from DCIS to invasive carcinoma, the stromal compartment demonstrates significant gene-expression change at this transition point. More specifically, whereas only three epithelial genes were differentially regulated at the transition from DCIS to invasive cancer, 76 stromal genes were upregulated and 229 stromal genes were downregulated at the identical transition point (93). To obtain an overview of the biological processes associated with these differentially expressed stromal genes at the preinvasive-to-invasive transition point, the authors (93) performed gene set enrichment analysis. The stromal genes that were differentially expressed at this transition demonstrated the highest correlation with gene sets that featured the components of the extracellular matrix and the *MMPs* responsible for matrix remodeling. At an individual gene level, *MMP2*, *MMP11* and *MMP14*, showed significant increased expression in IDC as compared with DCIS. These findings support the notion that stroma-produced *MMPs* may be key players driving the DCIS-to-IDC transition.

Taken together, these gene-expression and epigenetic data support the view that the tumor microenvironment is an important coconspirator rather than a passive bystander during breast carcinogenesis. These results also suggest that the tumor microenvironment participates in tumorigenesis even before tumor cells invade into the stroma and that it may play a critical role in the transition from preinvasive to invasive growth.

THE TRANSITION FROM PREINVASIVE TO INVASIVE BREAST CANCER

The watershed event in breast cancer progression is the transgression of tumor cells of the preinvasive stage of DCIS through the basement membrane into the surrounding stromal compartment. This transition is poorly understood, and exploring the molecular events that drive this transition continues to be of great interest. Phenotypic, genetic, and epigenetic changes have been detected in the neoplastic epithelium of the preinvasive DCIS stage of breast cancer progression. However, stage-specific molecular alterations within the neoplastic epithelial cells have not been identified in human breast cancer samples (93-95, 127, 138). Multiple experimental lines of evidence have highlighted the potential importance of molecular alterations in the nonneoplastic cells of the tumor microenvironment, rather than in neoplastic epithelial cells, during the transition from invasive to metastatic breast cancer (132, 141-144). Recently, Hu et al. (145) provided experimental evidence to support a similar phenomenon in the DCIS-to-IDC transition. Using a cell line model for DCIS (146) known as MCFDCIS, the authors demonstrated that the spontaneous transition from DCIS to IDC is not associated with additional molecular alterations within the neoplastic epithelial cells (145). Instead, Hu et al. showed that the DCIS-to-IDC transition is promoted

by fibroblasts and suppressed by myoepithelial cells that constitute the nonneoplastic stromal and intraluminal microenvironment of DCIS (145). The authors provided evidence for extensive cross talk among the TGF- β , Hedgehog, cell adhesion, and p63 signaling pathways in the MCFDCIS cells, which results in the loss of myoepithelial differentiation and accelerated progression to invasive disease (145).

On the basis of these recent molecular genetic data, our view of breast cancer progression as an epithelial-centric driven process has evolved, and two models of the DCIS-to-IDC transition have been proposed (147). First, the so called escape model emphasizes the role of neoplastic DCIS epithelial cells and proposes that genetic changes in combination with clonal selection give rise to a subpopulation of neoplastic cells with the ability to disrupt the myoepithelial layer, degrade the basement membrane of the duct, and invade into the surrounding stromal tissue. The so called release model, however, hypothesizes that alterations in the in the DCIS microenvironment such as phenotypic alterations of myoepithelial, myofibroblastic and fibroblastic cells and infiltration of inflammatory cells lead to the degradation of the basement membrane with subsequent invasion of the neoplastic epithelial cells. Current evidence supports a combination of the two models, in which changes in both the neoplastic epithelial cells and the nonneoplastic myoepithelial and stromal cells result in a tumor microenvironmental signaling network that ultimately facilitates the transition from preinvasive to invasive breast cancer (**Figure 7**).

MOLECULAR PROGNOSTICS FOR PREINVASIVE BREAST CANCER

Given that a minority (~15-30%) of women diagnosed with pure DCIS develop a subsequent breast tumor event within the first decade after treatment with lumpectomy, and that a majority (~70%) of women with pure DCIS are treated with lumpectomy in conjunction with radiation and antihormonal treatment, it is likely that many women with pure DCIS are being overtreated (148, 149). Thus, there is an unmet clinical need to identify a prognostic biomarker to accurately predict the clinical behavior of DCIS. Identification of such a biomarker will assist in selection of the most appropriate treatment regimen. Those patients expected to develop indolent disease could be treated with lumpectomy alone, whereas those expected to develop aggressive disease could be treated more aggressively.

Immunohistochemical and gene-expression profiling has confirmed the presence of molecular subtypes in DCIS that parallel the distinct molecular subtypes observed in invasive breast cancer (25, 91-93). The use of molecular profiling technologies to identify distinct features that predict the future behavior of invasive disease is well documented. However, the application of such approaches to the identification of molecular predictors of clinical behavior (i.e. invasive recurrence) of preinvasive disease has been hampered by several problems. First, because preinvasive disease is frequently microscopic in size, all of the tissue is processed through use of standard pathological formalin-fixed paraffin-embedded (FFPE) processes and utilized for clinical diagnostic purposes. Second, standard FFPE processes pose a significant technical challenge for high-throughput array-CGH and gene-expression microarray profiling. Third, and most importantly, large clinical cohorts and clinical trials of preinvasive disease with well-annotated clinical samples and long (10-20 years) clinical follow-up are lacking.

Recently, Gauthier et al. (149) used a knowledge-based approach to study the relationship of stress-induced senescence with progression of the basal subtype of DCIS to invasive breast cancer. The authors demonstrated that DCIS with high p16 and/or high COX2 in the absence of cell proliferation reflects a normal protective stress-activation response, that and this phenotype is associated with disease-free progression (149). However, DCIS with high p16 and/or high COX2 in the presence of high cellular proliferation was shown to reflect an abrogated (or abnormal) response to cellular stress, and this phenotype is associated

progression of DCIS to invasive breast cancer (149). This biomarker may represent a defining biological signature in the progression pathway of basal-like breast cancers, and could provide a useful tool in the clinical management of patients with basal-like DCIS. These preliminary results generate optimism that prognostic biomarkers of the different subtypes of DCIS exist, and identification of such biomarkers will further improve the clinical management of patients diagnosed with preinvasive breast cancer.

Conclusions

Over the past decade, translational and basic science researchers have witnessed many new approaches to the study of the invasive and preinvasive stages of breast cancer and to breast cancer progression. Significant advances in our knowledge of the molecular biology of invasive breast cancer have allowed us to better understand the heterogeneity of this disease as it relates to its biological and clinical behavior. Such advances have resulted in the development of prognostic and predictive molecular assays that are currently used in the management of patients with invasive breast cancer. Through combined use of advanced microdissection and ex vivo isolation techniques with high-throughput genomic, gene-expression and epigenetic technologies, research efforts have begun to piece together the complex molecular genetic and molecular biological interrelationship between preinvasive stages of breast cancer and invasive breast cancer. Preinvasive breast cancer appears to arise from two distinct molecular genetic evolutionary pathways. However, despite evidence for the existence of two evolutionary pathways, preinvasive breast cancer constitutes a spectrum of lesions with heterogeneous biology and clinical behavior. Thus, the next challenge facing breast cancer researchers is the successful integrating our knowledge of the molecular biology of preinvasive breast cancer to identify predictive biomarkers of disease-progression risk.

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Acronyms

FEA	flat epithelial atypia
ADH	atypical ductal hyperplasia
DCIS	ductal carcinoma in situ
IDC	invasive ductal carcinoma
ALH	atypical lobular hyperplasia
LCIS	lobular carcinoma in situ
ER	estrogen receptor
PR	progesterone receptor
HER2	Human Epidermal growth factor Receptor 2
MMP	matrix metalloproteinase

Mini-glossary

Preinvasive breast cancer	a clonal proliferation of epithelial cells confined within the breast ducts without penetration through the basement membrane and into stroma
Terminal ductal lobular unit (TDLU)	is the functional unit of the breast composed of a terminal duct and a lobule that consists of a group of acini
Ductal carcinoma in situ (DCIS)	the last stage of preinvasive ductal cancer, consists of a clonal proliferation of epithelial cells that appear malignant and accumulate within the lumens of the breast ducts
Atypical ductal hyperplasia (ADH)	intermediate stage of preinvasive ductal cancer, consists of a clonal proliferation of epithelial cells that possess some but not all of the features of DCIS.
Flat epithelial atypia (FEA)	earliest stage of preinvasive ductal cancer, consists of a minimal clonal proliferation of epithelial cells
Lobular carcinoma in situ (LCIS)	a clonal proliferation of acinar-like epithelial cells that appear malignant and accumulate within and distend the acini of a terminal ductal lobular unit.
Atypical lobular hyperplasia (ALH)	a clonal proliferation of breast acinar-like epithelial cells possessing some but not all the features of LCIS.
Tumor grade	is an important histopathological measurement of cellular differentiation and correlates with the clinical behavior of the tumor.
Gene-expression signature/profile	the expression of a set of genes that is associated with a distinct clinical/or biological phenotype

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SUMMARY POINTS

1. The ductal (FEA, ADH and DCIS) and lobular (ALH and LCIS) preinvasive stages of the breast cancer are nonobligate precursors to invasive disease. These lesions constitute a spectrum of morphological entities with variable clinical behavior.
2. Genetic and gene-expression studies reveal two distinct molecular pathways of breast cancer progression that, in general, correlate with the histopathological parameter of tumor grade, resulting in a contemporary model of breast cancer progression that also accounts for intrastage heterogeneity.
3. Distinct genetic, epigenetic and gene-expression alterations occur in breast epithelium at the transition from normal to preinvasive breast cancer. However, additional molecular changes appear to be infrequent at the transition from preinvasive to invasive disease. The latter observation suggests that preinvasive epithelium possess the molecular machinery for aggressive behavior and that progression may depend upon other factors, such as quantitative levels and timing of molecular events.
4. The tumor-stromal and myoepithelial microenvironments demonstrate unique epigenetic and gene-expression alterations at the normal-to- preinvasive transition as well as at the preinvasive -to-invasive transition.
5. Recent gene-expression and epigenetic data strongly suggest that the stromal and myoepithelial microenvironment of preinvasive breast cancer actively participates in the transition from preinvasive to invasive disease. Current data point to a complex stromal-epithelial signaling interplay between matrix remodeling (e.g., secretion of MMPs and cathepsins by stromal and myoepithelial cells) and signal transduction pathways linked to growth and migration (e.g., secretion of CXCL12, CXCL14, SFRP1 and WIF1).
6. Translational research over the past two decades has resulted in the successful application and use of molecular diagnostics in the clinical management of invasive breast cancer. Although application of molecular diagnostics to preinvasive breast cancer lags behind its invasive counterpart, the recent discovery of a p16- and COX2-based prognostic signature in basal-like DCIS has generated optimism that clinically useful molecular biomarkers of preinvasive breast disease will be a reality in the near future.

FUTURE DIRECTIONS

1. Identification of the precise cell or cells of origin responsible for the preinvasive stages of breast cancer would provide a critical starting point for a better understanding of the molecular events underlying the development of human breast cancer.
2. Because the transition from preinvasive cancer to invasive cancer is the critical watershed event in breast cancer progression, a more complete understanding of the basic mechanisms underlying breast cancer invasion is needed.
3. Unraveling the complex heterotypic signaling in the microenvironment of preinvasive breast cancer will require the development of improved in vivo and in vitro models of breast cancer progression. More specifically, it will require the development of models that represent the various subtypes (ER+/HER-, ER+/HER+, ER-/HER+, and ER-/PR-/HER-) of preinvasive disease, as each subtype is associated with distinct molecular biological pathways and clinical behavior.
4. Preinvasive breast cancer constitutes a spectrum of lesions with heterogeneous biology and clinical behavior. Currently, it is not possible to specify with certainty which preinvasive lesion will progress to invasive disease and which will not. Thus, there is a critical need to develop a molecular classification system or to identify biomarkers for preinvasive breast cancer that will accurately predict risk of local recurrence and, more importantly, risk of subsequent invasive disease.
5. Identification of prognostic (prediction of local recurrence and invasion) and treatment-predictive biomarkers for preinvasive breast cancer will require the establishment of a large international cohort of patients with long-term clinical follow-up data linked to pathology tissue repositories.
6. Development and implementation of novel molecular-based imaging reagents and methods for the detection of microscopic preinvasive breast cancer will be of tantamount importance, as they will allow for potentially curative therapeutic intervention.

HISTOPATHOLOGICAL GRADING

Invasive and in situ breast cancers display enormous histological diversity; they range from well-differentiated, slow-growing tumors to poorly differentiated, rapidly growing tumor. Given this diversity, investigators have proposed multiple histological grading schemes to subclassify invasive and in situ carcinomas into clinically meaningful prognostic categories. Histological grading describes the microscopic growth pattern of breast cancer as well as the cytological features of differentiation. Pathologists employ different grading to evaluate invasive and in situ carcinomas. For IDCs, the most commonly accepted histomorphological grading system—the modified Bloom-Richardson system—evaluates three distinct tumor factors, namely tubule formation (glandular differentiation), nuclear pleomorphism (a reflection of DNA content), and mitotic rate (cellular proliferation). This grading system also classifies breast tumors into three distinct categories: low-, intermediate-, and high-grade malignancies. For in situ ductal carcinomas, a number of DCIS grading schemes have been proposed that use various measurements of architectural patterns (cellular polarization), nuclear morphology, mitosis, and necrosis. In general, for both invasive and in situ cancers, a low grade (grade I) tumor consists of malignant epithelial cells that lack significant nuclear pleomorphism and mitotic activity and that form glandular (acinar-like) structures. At the opposite extreme, a high grade (grade III) tumor consists of malignant cells possessing marked nuclear pleomorphism and high mitotic activity that completely fail to form glandular elements and that often exhibit necrosis. Clinically, it is well established that high- and low-grade tumors are associated with the highest and lowest rates of recurrence and the shortest and longest recurrence times. Intermediate-grade breast cancers display phenotypic and clinical behavioral characteristics that lie in between low- and high-grade tumors, and it is this group of tumors that poses the greatest interobserver grading challenge for pathologists and the greatest treatment challenge for oncologists. However, as described in the main text, gene-expression and genomic data suggest that the group of intermediate-grade tumors consists of either low grade-like or high grade-like tumors, and that DCIS and IDCs should be classified into two, rather than three, biologically and clinically meaningful categories.

NATURAL HISTORY OF PREINVASIVE BREAST CANCER

Preinvasive breast cancer constitutes approximately one third of all newly diagnosed breast cancer in the United States. Although women do not die of preinvasive disease, it is well known that a subpopulation of these women will subsequently develop invasive cancer. Women diagnosed with ADH and ALH demonstrate a 3.5- to 5.0- fold-increased risk of developing invasive breast cancer compared with the general population. The development of invasive breast cancer in women diagnosed with LCIS is estimated at 7-9 times the relative risk of women in the general population, whereas women diagnosed with DCIS have an estimated 4-12 times relative risk of developing invasive breast cancer. In general, the degree of relative risk directly correlates with biologically aggressive features that are captured by tumor grade: for instance, low grade in situ carcinomas tend to have a better prognosis than their high grade counterparts.

Figure 1.

Hypothetical models of the origin of human breast cancer. Based on the stochastic model of breast carcinogenesis (*a*), any epithelial cell type (e.g. stem cell, progenitor, or differentiated cell) may be the target of the initiating event. Each breast cancer subtype is initiated in a different cell type. In the cancer stem cell model (*b*), the cell of origin can be the same stem cell or progenitor cell for the different subtypes. The tumor phenotype is determined by a combination of genetic and epigenetic events. Abbreviations: ER+(-), presence (absence) of immunohistological expression or *HER* gene amplification; PR+(-), presence (absence) of immunohistochemical expression of progesterone receptor (PR) expression.

Figure 2.

Microanatomy of the terminal duct lobular unit (TDLU). (a). Low-power photomicrograph of a hematoxylin and eosin (H&E)-stained tissue section of human breast with several TDLUs, each consisting of a cluster of acini (ductules) and a terminal duct (TD). High-power images of H&E-stained (b,c) and calponin-immunostained (d,e) tissue sections demonstrate the two cell layer anatomy of the acini (b,e) and the TD of the TDLU. Both the acini and TD display a continuous outer layer of myoepithelial cells (ME) that are immunoreactive for calponin (d,e, *brown elongate cells*). The ME surround the cuboidal or the low columnar luminal epithelial cells (LE) of the acinus and TD, respectively. The basement membrane (BM) surrounds the ME. The TDLU is surrounded by the breast stromal (S) compartment.

Figure 3.

Histomorphological classification of preinvasive and invasive breast cancer. The preinvasive stages of the lobular type consist of atypical lobular hyperplasia (**ALH**) and lobular carcinoma in situ (**LCIS**).

Differentiation of ALH from LCIS is based upon the extent of proliferation and distension of the acini within the terminal duct lobular unit (TDLU). Pleomorphic LCIS differs from co-called classic LCIS in that the pleomorphic type consists of a proliferation of cells that are highly variable in size and shape and loosely cohesive, with or without necrosis, whereas the classic type consists of a proliferation of uniform, small, loosely cohesive, nonpolarized epithelial cells. The epithelial proliferation associated with preinvasive LCIS is confined to the ductal system, whereas invasive lobular carcinoma (ILC) infiltrates through the basement membrane into the surrounding stroma. The preinvasive stages of ductal type consists of flat epithelial atypia (FEA), atypical ductal hyperplasia (ADH), and ductal carcinoma in situ (DCIS). FEA is characterized by a minimal proliferation of native luminal cells of the TDLU by one to several layers of monomorphic cuboidal or columnar epithelial cells with low-grade cytological atypia. ADH differs from FEA in that in ADH the cells grow in a pseudostratified manner with secondary architectural atypia in the form of micropapillae, Roman arches (*arrow*) and trabecular bars. The differentiation of ADH from DCIS is based on the degree of architectural atypia and on the size and the extent of epithelial proliferation. Low-grade DCIS consists of small, cohesive, polarized, uniform cells of low proliferative capacity while high-grade DCIS consists of large, pleomorphic cells of high proliferative capacity with necrosis (*asterisk*). Intermediate-grade DCIS

consists of small- to medium-sized, polarized cells with moderate nuclear pleomorphism and low proliferative capacity with or without necrosis (*asterisk*). Preinvasive carcinoma of the ductal type (e.g.; DCIS) consists of proliferation of medium- to large sized polarized cells confined to the ductal system whereas invasive ductal carcinoma (IDC) is characterized by the growth and invasion of neoplastic cells beyond the basement membrane and into the surrounding stroma.

“Classic” (Wellings) Model of Progression

“Alternative” Model of Progression

Figure 4.

Traditional linear models of breast cancer progression. (a) Classic and (b) alternative linear multistep models of human ductal breast cancer progression. The two models are identical, with the exception of the placement of usual ductal hyperplasia (UDH) as the precursor to atypical ductal hyperplasia (ADH). The classic model was initially based on histomorphological observations, whereas the alternative model was initially proposed based upon the epidemiological observations. Recently, multiple lines of evidence (histomorphological, immunohistochemical, and molecular genetic) support the classic model and contest the alternative model of progression. Molecular alterations occurring in normal breast epithelium result in flat epithelial atypia (FEA). FEA leads to additional changes give rise to ADH and ductal carcinoma in situ (DCIS), upon which subsequent genetic and epigenetic alterations in turn give rise to invasive ductal carcinoma (IDC).

Figure 5.

Molecular classification of invasive ductal breast cancer. Genetic and gene-expression data classify invasive ductal carcinomas into two distinct molecular biological and clinicopathological pathways. (a) The low grade-like gene-expression molecular pathway is characterized by chromosome 16q loss, predominant estrogen receptor (ER) and progesterone receptor (PR) expression (ER+, PR+) and a low-grade gene-expression profile populated with genes associated with ER positivity (ER+). (b) The high grade-like gene-expression molecular pathway is characterized by loss of chromosome 13q, gain of 11q13 and/or amplification of 17q12, infrequent expression of ER and PR, and a high grade-like gene-expression signature populated with genes associated with cell cycle, centrosomal function and DNA repair. The low-grade tumors express a unique set of genes that are rarely expressed in high-grade tumors, and vice versa. The luminal A and luminal B subtypes constitute the majority of invasive breast cancers in the low grade-like gene-expression molecular pathway and generally display indolent clinical behavior, whereas the human epidermal growth receptor 2 (HER2) and basal subtypes constitute the majority of cancers in the high grade-like pathway and generally display an aggressive clinical behavior. Light blue rectangles denote morphological ductal subtypes. Abbreviations: HER+(-), presence (absence) of immunohistological expression or HER gene amplification; IDC, invasive ductal carcinoma; -16q, loss of chromosome 16q; +1q, gain of chromosome 1q; -13q, loss of chromosome 13q; +11q13, gain of chromosomal region 11q13; +17q12, amplification of chromosomal region 17q12.

Figure 6.

Contemporary multistep, two-dimensional model of human breast cancer progression derived from morphological, immunohistochemical, genetic and gene-expression data. Distinct molecular events occur in normal breast epithelium, giving rise to two distinct divergent molecular pathways within which linear pathological stage progression (*horizontal black arrows*) and intrastage heterogeneity (i.e., tumor-grade evolution; *vertical red dashed arrows*) occur. (a) The low grade-like gene-expression molecular pathway is characterized by chromosome 16q loss, predominant estrogen and progesterone receptor expression (ER+, PR+) and a low-grade gene-expression profile populated with genes associated with ER positivity (ER+). This low-grade pathway is observed in preinvasive lesions of both the ductal subtype (*light blue rectangles*) and lobular subtype (*green rectangles*). (b) The high grade-like gene-expression molecular pathway is characterized by loss of chromosome 13q; gain of 11q13 and/or amplification of 17q12; infrequent expression of ER and PR; and a high grade-like gene-expression signature populated with genes associated with cell cycle, centrosomal function, and DNA repair. Although pleomorphic atypical ductal hyperplasia (ALH), pleomorphic lobular carcinoma in situ (LCIS), and pleomorphic invasive lobular carcinoma (ILC), phenotypically resemble high grade tumors, immunohistochemical (ER positivity) and genetic (16q loss and 1q gain) data support an evolutionary association with the low grade-like gene-expression molecular pathway. Recent immunohisto-chemical, morphological and genetic data support the concept of intrastage tumor-grade progression (*red dashed arrows*), which probably accounts for the observation of intratumoral heterogeneity. Light blue and green rectangles denote ductal and lobular morphological

subtypes, respectively. Abbreviations: ALH, atypical lobular hyperplasia; DCIS, ductal carcinoma in situ; FEA, flat epithelial atypia; HER+(-), presence (absence) of immunohistochemical expression or *HER* gene amplification; IDC, invasive ductal carcinoma; -16q, loss of chromosome 16q; +1q, gain of chromosome 1q; -13q, loss of chromosome 13q; +11q13, gain of chromosomal region 11q13; +17q12, amplification of chromosomal region 17q12.

Figure 7.

Tumor-stromal interactions during the preinvasive-to-invasive breast cancer transition. Multiple lines of evidence demonstrate marked genetic, epigenetic and gene-expression alterations in the neoplastic epithelium of preinvasive breast cancer as compared with normal breast epithelium. At the transition from preinvasive to invasive breast cancer, the neoplastic epithelium is not associated with additional qualitative molecular alterations. Instead, the nonneoplastic tumor microenvironment cells, in particular myoepithelial cells and stromal fibroblasts, myofibroblasts, and leukocytes, display significant molecular alterations that are associated with and may contribute to the transition from in-situ to invasive breast carcinoma. In preinvasive breast cancer (such as that illustrated in this hematoxylin and eosin-stained section), overexpression of cytokines and chemokines (as compared with normal breast epithelium) by neoplastic epithelium can induce an autocrine pathway that directly stimulates tumor growth. These tumor epithelial cell-derived cytokines can also induce several paracrine pathways. In the first paracrine pathway, tumor epithelial cell-derived cytokines and chemokines induce decreased stromal expression of growth pathway antagonists (i.e. WIF1 and SFRP1) that, in turn, favors tumor epithelial cell growth. Furthermore, the tumor epithelium can induce stromal cell expression of proteases [matrix metalloproteinases (MMPs) and cathepsins] that are involved in extracellular matrix remodeling, angiogenesis and basement membrane (*black arrowheads*) degradation. In the second paracrine pathway, epithelial cytokines induce myoepithelial cells to secrete

proteases (MMPs) that degrade the basement membrane, thereby facilitating the transition from in situ to invasive carcinoma, and cytokines (CXCL12, CXCL14) that promote tumor epithelial cell growth, migration, and invasion. Abbreviation: TGF- β , transforming growth factor beta,