Normal Breast Development

Studies of breast cancer from the 1970s to the mid-1990s focused mainly on changes in breast cancer with little regard for normal tissue or development. A lack of knowledge of normal mammary gland development and function limited the understanding of tumor-specific changes. In 1998, the NCI-directed Breast Cancer Progress Review Group stated, “Our limited understanding of the biology and developmental genetics of the normal mammary gland is a barrier to progress.”1 This statement led to a major increase in such studies, with mouse models giving invaluable insights into the molecular biology of both normal mammary gland development and breast cancer.2 Extensive genetic and molecular analysis of mammary gland development in small and large animals has rapidly defined many of the intricate molecular networks, such as interactions between steroid hormone and growth factors, that are critical for all stages of development and function.3 Intriguingly, many of these same pathways have major roles in breast cancer development and progression and thus are major therapeutic targets.4 One of the greatest advances has been the recent identification and characterization of mouse mammary stem cells. Sorting cell populations using cell surface markers has shown that the myoepithelial cell layer contains adult mammary stem cells and that a single cell transplanted into the mouse can produce every epithelial cell of the mammary gland.5,6 Evidence that mammary stem cell number and function are regulated by hormones such as progesterone and RANK ligand is consistent with the major functions of hormones in mammary gland function and may have implications for human breast cancer development and treatment.7,8 Intriguingly, BRCA1 has also been found to regulate mammary stem cell number and function,9 and evidence suggests that BRCA1 mutations may arise from a blockade of progenitor cell development.10

Clonal and Stem Cell Hypotheses

There are two main models for cancer initiation and progression, the clonal evolution hypothesis11 and the cancer stem cell (CSC) hypothesis.12 In the clonal evolution hypothesis, any cell is susceptible to sporadic random mutation, and a particular combination of mutations allows selection of a cell to evolve to become immortal and tumorigenic. Thus any cell within a tumor can maintain tumorigenesis. In contrast, the CSC hypothesis posits that only stem and progenitor cells, which are a minor fraction of cells within a tumor, can give rise to self-renewing tumor cells. These two hypotheses have major implications for understanding breast cancer development and therapeutic intervention.13 However, the two hypotheses both rely on major assumptions that are virtually impossible to assess, given the inherent difficulty in tracking cell transformation and differentiation in human breast tumors. Although both hypotheses are often presented as competing ideas, it is highly likely that tumorigenesis is a combination of both models.14 Future studies will be required to define cancer cell growth and differentiation and better define the role of clonal and cancer stem cell function, as this will likely have major implications for the prevention and treatment of the disease.

Preneoplastic Progression

Advances in molecular biology have had a major impact in the understanding of premalignant progression. Early studies using anatomic pathology and epidemiology revealed that certain premalignant breast lesions such as atypical ductal hyperplasia (ADH) and ductal carcinoma in situ (DCIS) were associated with an increased risk of developing subsequent invasive ductal cancer (IDC).15 Analysis of changes in DNA copy number and loss of heterozygosity showed that synchronous and metachronous DCIS and IDC showed almost identical genetic changes indicating...
that DCIS is the precursor for IDC. Consistent with this, the diversity of transcriptomic change and IDC subtypes is similarly found in DCIS. Although 80% to 90% of breast cancer is of ductal origin, a smaller percentage (but still large number of breast cancers) are of lobular origin. These are much less studied; however a similar pattern of progression from atypical lobular hyperplasia to lobular carcinoma in situ to invasive lobular cancer is thought to occur.

**Pathophysiology and Risk Factors**

**Genetics and Family History**

Approximately 5% to 10% of breast cancer cases have a familial or hereditary component. Advances in molecular biology have had a profound effect on the diagnosis, risk reduction, and treatment of hereditary breast cancer. Classic genetic mapping and cloning studies identified mutations in two genes, BRCA1 and BRCA2, that account for the majority of hereditary breast cancer. Screening for mutations in BRCA1 and BRCA2 is now standard of care in women with clinical features suggestive of hereditary breast cancer, and recent advances in DNA sequencing permit advanced screening at a reduced price. BRCA1 and BRCA2 are large multifunctional proteins that have a major role in DNA repair. Genetic deletion of these genes in mice and in cells results in genomic instability and sensitivity to transformation. Mutations in other genes such as BRIP1, RAD51, CHEK2, ATM, and PALB2 are also associated with hereditary breast cancer, and importantly, these genes also participate in DNA repair, indicating that loss of DNA repair is a major contributor to familial breast cancer.

The most recent clinically relevant advance to come from understanding the molecular biology of BRCA1 and BRCA2 function has been the finding that cells with mutations in these genes are sensitive to blockade of polyADP ribose polymerase (PARP) and the alternative base-excision DNA repair (BER) pathway. This finding is founded on the genetic precept of synthetic lethality, whereby a cancer cell with wild-type BRCA1 can compensate for inhibition of BER by using the classical homologous recombination pathway. In contrast, cells with loss of BRCA function have no pathway to overcome the loss of BER and thus undergo cell death. The result is a dramatic relative increase in sensitivity to PARP inhibition in cells with loss of BRCA. This concept of synthetic lethality has led to clinical trials showing dramatic responses to PARP inhibitors in cancer patients with germline mutations in BRCA1 or BRCA2.

**Hormones**

Many risk factors for breast cancer are related to estrogen exposure. These include age at menarche and menopause, parity and age at first-time pregnancy, and breast feeding. The common link among these factors is the number of menstrual cycles that women experience, and thus it is hypothesized that cumulative length of exposure of the breast to sex hormones may increase risk of breast cancer. These data are supported by evidence that surgical or pharmacological suppression of ovarian function significantly reduces breast cancer risk, and prolonged postmenopausal use of combined estrogen and progesterone therapy increases risk.

**Environment**

Although there are clear geographic differences in the rate of breast cancer, tying these different rates of breast cancer to specific environmental exposures (defined broadly) has been challenging. For example, there is a strong association between risk of breast cancer and fat intake across countries; however, direct proof that fat intake correlates with risk of breast cancer has been challenging to obtain. A recent comprehensive review by the Institute of Medicine reported that alcohol consumption, postmenopausal weight gain, smoking, lack of exercise, and hormone replacement therapy are associated with increased risk of breast cancer. There is also evidence for increased risk with exposure to benzene, 1,3-butadiene, ethylene oxide, chemical pollutants in vehicle exhaust, gasoline fumes, and smoking. Recent studies have begun to link risk factors to specific etiologies of breast cancer and potentially highlight the different etiologies of breast cancer subtypes. A greater understanding of the molecular biology of breast cancer initiation and progression is likely to provide greater clarity about breast cancer risk factors.

**Molecular Subtyping**

**Histopathology and Molecular Pathology**

Multiple lines of evidence demonstrate that breast cancer is not a single disease, but a mixture of different subtypes. Within these subtypes, there exists further significant diversity. By histopathology, the majority of breast cancers are invasive ductal cancer (IDC) (about 75%), invasive lobular cancer (ILC) (about 10%), or mixed IDC/ILC (about 5%). Minor populations are mucinous, tubular, medullary, papillary, and metaplastic breast cancers. Histopathology
has been very useful to define these subtypes and then further assess tumor aggressiveness by measures such as tumor grade. Molecular analysis of breast cancers allows further subclassification of the major subtypes, such as IDC, into subtypes with different outcome. The first biomarker, initially discovered and studied more than 40 years ago, was the estrogen receptor alpha (ER). ER-positive breast tumors generally have a somewhat better prognosis, and patients with these tumors are candidates for antihormone therapy. ER-positive tumors can be further subdivided by levels of the estrogen-inducible gene progesterone receptor (PR), with loss of PR indicating lack of ER action and poor outcome.

The second subclassification came with the discovery of ErbB2 (HER2) amplification. Approximately 20% of IDC have amplification and overexpression of HER2. Patients with HER2-positive tumors have a poorer natural history, but many respond to anti-HER2 therapy. Tumors that lack expression of ER, PR, or HER2 have been termed triple-negative breast cancer (TNBC). There has been intense study of this subset of breast cancer in the past 10 years as they have poor outcome and are insensitive to targeted therapies such as antihormonal or anti-HER2 therapy.

Transcriptomics

The ability to undertake simultaneous measurement of thousands of genes, via microarray technology, allowed a fundamental shift in the study of the molecular biology of breast cancer. The information provided a much finer delineation of breast cancer subtypes than that afforded by histopathology, and also gave insight into the biological underpinning that highlights potential new therapeutic targets. Perou and colleagues performed the first microarray analysis of human breast cancer and identified a set of “intrinsic” genes that defined five major subtypes (luminal A, luminal B, normal-like, HER2-enriched, and basal-like) with different outcomes. These results have remained highly reproducible. A major breakthrough in these studies was the push to make these large datasets publicly available, which greatly facilitated breast cancer research via in silico analysis.

Many resources are available for the analysis of large publicly available datasets of breast cancer (e.g., Oncomine.org; gene expression omnibus GEO—www.ncbi.nlm.nih.gov/geo).

From these analyses has come the understanding that luminal tumors express ER and ER-regulated genes. Luminal A tumors tend to have low expression of proliferation genes and have a very good prognosis, whereas Luminal B tumors have lower levels of ER and ER-regulated genes, exhibit markedly higher proliferation and mutation of p53, and have a worse prognosis. Some luminal B tumors are ER+/HER2+. The HER2+ subclass contains tumors that have high levels of HER2 and HER2-regulated genes. A recent integrated analysis of copy number aberrations (CNA) and gene expression showed that the HER2 subtype is largely regulated by the amplification of HER2 (in a cis manner) rather than amplification of other genes that act to increase HER2 expression (in a trans manner).

A large subset (approximately 80%) of TNBC express genes associated with basal/myoepithelial breast cells (such as cytokeratin 5) and has been termed basal-like breast cancers. It should be noted that basal-like tumors are not synonymous with TNBC but represent a subset of TNBC.

The normal-like classification of breast cancer is controversial. These tumors account for approximately 5% to 10% of breast cancer and show gene expression profiles similar to normal breast tissue or fibroadenomas such as adipose genes. Many investigators believe that the normal-like subtype is an artifact of low tumor cellularity. Indeed, studies using gene expression profiling of microdissected breast cancer cells do not identify the normal-like subtype. Similarly, gene expression profiling of breast cancer cell lines also does not identify normal-like cell lines. However, the identification of this subtype does highlight the issue of tumor heterogeneity and cellularity when performing microarray analysis of breast cancers. Although tremendous advances have been made in microarray profiling, most of these profiles represent an average of gene expression across multiple cell types within a tumor (including leukocytes, adipose, vascular cells, etc.). It is likely that new approaches, such as single cell transcriptomic profiling, will provide a new level of detailed insight into breast cancer molecular biology.

Although microarray technology allowed genome-wide analysis of mRNA levels, recent advances in massively parallel sequencing of RNA are giving new insight into not only RNA levels, but also changes in RNA splicing and polyadenylation usage and noncoding RNAs in breast cancer. In addition, sequencing of two ends of RNA allows the identification of neo-RNA fusion genes that are generated by fusion of two RNAs. A recent comprehensive analysis by paired-end RNA-sequencing of 89 breast cancer cell lines and tumors identified 384 expressed fusion RNAs. However, only one (SEC16A-NOTCH1) was found in more than one tumor. Several genes appeared fused multiple times, but often with different partner genes. Overall this study highlighted the molecular diversity of human breast cancers and the complexity of targeting specific mutations.

Genomics

Genomic instability, the change in DNA structure and copy number, is a hallmark of virtually all breast cancers. Early studies of genomic change relied on cytogenetics and
fluorescence in situ hybridization (FISH) and revealed large chromosome changes and discrete copy number changes such as amplification of HER2. Microarray technology transformed the study of genomics by allowing genome-wide study using comparative genomic hybridization. Genomic instability permits changes in multiple genes required for cancer progression. Consistent with this, analysis of DNA copy number aberrations (CNA) during breast cancer progression shows a large increase in such changes at the transition from ADH to DCIS. Early studies identified recurrent oncogene-containing amplifications such as 8q12 (FGFR1), 8q24 (myc), 11q13 (CCND1), and 17q21 (ErbB2). However, these amplified regions often contain numerous genes that may play a role in breast cancer.

Several large studies of CNA in breast cancer have identified three major broad types. The first type, termed simple, exhibits few CNA and tends to have gain (1q and 16p) or loss (16q) of whole chromosome arms. This form of CNA tends to be associated with luminal A tumors with good outcome. The second type, termed amplifier or firestorm, is associated with focal high levels of amplification within a background of other complex gains and losses. A third type, termed complex or sawtooth, is associated with numerous low-level amplifications and losses across the genome. This pattern is most common in the aggressive TNBC and is associated with TP53 mutation. Many public resources of CNA in breast cancer are available in user-friendly formats such as Tumorscape (www.broadinstitute.org/tumorscape) or the UCSC Cancer genome browser (https://genome-cancer.ucsc.edu).

Several large consortia, including the Cancer Genome Atlas (TCGA), the International Cancer Genome Consortium (ICGC), and the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC), are examining genome-wide CNA and gene expression changes in large numbers of breast cancers. METABRIC recently reported a comprehensive analysis of 2136 cases of breast cancer and examined how CNA alters transcriptional profiles, in a cis- or trans-acting manner. Interestingly, they found that the HER2 and basal-like subtypes of breast cancer were most associated with the cis effects of CNA, implying that the genes that undergo CNA themselves tend to be drivers of the diseases rather than the gene products acting in a trans manner to activate other drivers of the disease. Perhaps most striking was the initial observation that an integrated analysis of both CNA and gene expression levels led to the identification of 10 subtypes of breast cancers, each having different outcomes. It will likely be many years before the full implication of datasets such as METABRIC are fully understood and translated into clinical care.

While assays for measuring CNA have rapidly advanced, analysis of structural DNA changes, such as inversions and translocations, has lagged behind. Indeed, high-throughput methods have only recently become available with the advent of massively parallel sequencing. Sequencing of paired ends of DNA allows determination of genomic structure. The initial comprehensive analysis of the MCF-7 cell line revealed numerous structural changes including translocations and novel fusion genes. Paired-end sequencing of other human breast cancers and cell lines unveiled patterns of translocations, and the greatest number of events was seen in aggressive TNBC. Sequencing of cell lines has provided many new insights, one of the most intriguing being chromothripsis, in which cancer genomes seem to have undergone a single catastrophic damaging event with numerous mistakes in the repair, resulting in a large-scale rearrangement of part of the genome. Although it is currently unclear how these complex rearrangements are generated, it is possible that they are due to replication-induced DNA damage and repair.

Determination of somatic base-pair mutations in candidate genes has been undertaken in breast cancers for many years; however, it is only recently that a comprehensive catalog has been obtained via massively parallel sequencing. A comprehensive database of somatic mutations in breast cancer can be found at COSMIC, the Catalog of Somatic Mutations in Cancer (sanger.ac.uk/genetics/CGP/cosmic). Similar to other cancers, TP53 is commonly mutated (23% of breast cancers), but the most frequently mutated gene is PIK3CA, the catalytic subunit of phosphatidylinositol-3′-kinase.

## Multiplexed Genomic and Transcriptomic Prognostic Tests

A dozen years have passed since the initial description of the five intrinsic subtypes of breast cancers, and many thousands of breast cancers have been subjected to microarray analysis. Although these studies have defined the transcriptional landscape of breast cancer and identified new therapeutic targets, translation of this work to clinical use has been slow. The first multigene assay to be used clinically was OncotypeDx (Genomic Health Inc, Redwood City, Calif). This is a RT-PCR test, based on the measurement of expression of 16 genes and 5 reference genes in ER+ node-negative disease, which can predict risk of recurrence on tamoxifen. More importantly, the test reclassifies patients at high risk of recurrence and results in identification of patients with disease with a low risk of recurrence and little benefit from adjuvant chemotherapy. A second microarray-based approach (MammaPrint) for determining breast cancer prognosis is based on the measurement of the expression of 70 genes (Agendia Inc, Irvine, Calif). It classifies breast cancers as either low or high risk.
Molecular Basis of Breast Cancer

ER Action

The steroid hormone estradiol signals through two related receptors, ERα and ERβ. Data from in vitro studies and mouse models, as well as studies using clinical specimens, have provided concrete evidence that ERα is the dominant regulator of both normal breast development and breast cancer. ERα (referred to hereafter as ER) is a nuclear hormone receptor that binds the estradiol with very high affinity. Ligand binding changes receptor conformation to allow binding to enhancer elements in DNA. Initial studies suggested that these DNA elements were close to promoters and provided a simple model of how ER directly affects promoter activity. However, advances in genome-wide DNA binding (e.g., ChIP-seq), chromatin conformation (e.g., Chia-PET), and RNA transcription assays (e.g., GRO-seq) have shown that ER action is much more complicated than previously thought.76,77 ER often binds hundreds of kilobases upstream of promoters to regulate transcription via looping of large segments of DNA. ERα’s action is regulated through its interaction with numerous co-regulatory proteins, which can either activate or repress its transcriptional activity.78 Several of those co-regulators have been shown to be associated with endocrine resistance, such as SRC1,79,80 SRC3,81,82 and NCoR1.83

Although ER clearly functions as a classical ligand-dependent DNA-binding transcription factor, it may also function in an extranuclear nongenomic manner.84 This is, at least in part, mediated via growth factor-activated signaling pathway, ultimately leading to phosphorylation of ER, especially at S118, S167, and S305, and subsequent recruitment of co-regulators and DNA binding.85 Although such a role is mechanistically attractive, especially with regard to ER being associated with metastatic processes through interaction with SRC, PI3K, and MAPK signaling, the clinical relevance of ER nongenomic action in breast cancer is controversial. A well-controlled large study using more than

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This table is not comprehensive, but provides an example of breast cancer multiplexed gene tests that are currently in development or have been commercialized. The type of sample, number of genes, indication, and ability to predict therapy may change based on commercial development. Prediction of response to therapy has been shown for many of the tests; however, for some of the tests this may not be used or approved as an indication for the test. Chemo, Chemotherapy; ER, estrogen receptor; FDA, U.S. Food and Drug Administration; FFPE, formalin-fixed paraffin-embedded; IVDMIA, In Vitro Diagnostic Multivariate Index Assay; RT-PCR, reverse transcriptase polymerase chain reaction; TAM, tamoxifen.

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Unfortunately, tumors can overcome this molecular switch, in part explaining its resistance in HER2-overexpressing tumors.87 A recent whole-genome sequencing of breast tumors before neoadjuvant therapy has revealed mutations in the TP53 pathway, and increased mutation of the TP53 pathway (38%) in aromatase inhibitor–resistant tumors compared to those that responded to therapy (17%).

Perhaps the most logical approach to blockade of ER action in breast cancer would be the total removal of ER protein such that no ligand-dependent or independent activation could occur. ICI 182780 (fulvestrant, AstraZeneca) is a selective estrogen receptor downregulator (SERD) that is similar in structure to estradiol, binds ER with the same affinity, and leads to rapid receptor degradation.96 The actual mechanism of degradation is unknown but likely involves the proteasome. Clinical development of fulvestrant has been hampered by the fact that it requires regular intramuscular injection, and early trials likely used doses (250 mg) that were not sufficient to saturate ER. A recent Phase II trial comparing first-line fulvestrant (500 mg) to the aromatase inhibitor, anastrozole, showed superiority in time to tumor progression for fulvestrant.97 Furthermore, anastrozole plus fulvestrant was recently reported to be superior to anastrozole alone.98 Further delineation of the molecular mechanisms whereby ER activates gene transcription, and how SERMs and SERDs inhibit ER activity, will likely lead to the development of improved anti-ER therapies that minimize the emergence of therapeutic resistance.

Chromatin Remodeling

It is now commonly accepted that epigenetic changes such as DNA methylation, chromatin changes, and regulation of gene expression by miRNA play a role in carcinogenesis in many tumors, including breast cancer.99 Aberrant DNA methylation has been studied extensively, both at the single gene and genome-wide levels. A number of genes have been reproducibly shown to be methylated in breast tumors, such as RASSF1A, PR, RARβ, CCND2, and BRCA1. However, at this point, no predictive or prognostic marker includes measurement of methylation.100 Unexpectedly, sequencing studies of tumors have revealed very frequent somatic mutations in chromatin-modifying genes, including in the family of ATP-dependent chromatin remodeling proteins, enzymes modifying posttranslational modification of histones, and histone variants. For example, mutations in the H3K4 methyltransferase MLL are among the most frequent in breast tumors.63,101 Other histone-modifying enzymes are highly expressed in aggressive breast tumors, such as the H3K27 methyltransferase EZH2, which was also shown to contribute to the expansion of progenitor cells.102 It is therefore not surprising that there are many efforts to target deregulated epigenetic pathways in breast cancer. In contrast to hematopoietic malignancies, there are currently no approved breast cancer epigenetic therapies. However, trials are ongoing with drugs that inhibit HDACs and DNA
methyltransferases as well as efforts to target other histone-modifying enzymes, such as EZH2.99

Growth Factors

Growth factors play a major role in both mammary gland development and breast cancer and have been studied intensely as therapeutic targets.103 The best studied growth factor receptor in breast cancer is HER2 (ErbB2). HER2 is amplified in approximately 20% of breast cancers, and its amplification and/or overexpression is associated with poor prognosis.104 HER2 is a member of the larger HER/ErbB family consisting of epidermal growth factor receptor (EGFR/ErbB1/HER1), ErbB3/HER3, and ErbB4/HER4. Amplification of HER2 is thought to cause increased homo- and heterodimerization with other family members, resulting in constitutive activation of downstream signaling pathways leading to cancer cell growth and survival. The identification of this dominant activating oncogene led to one of the first examples of bedside-to-bench translational research with the development of monoclonal antibodies that block HER2. Trastuzumab (Herceptin, Genentech, South San Francisco, Calif) is a humanized monoclonal antibody that binds the extracellular domain of ErbB2. Trials of trastuzumab plus chemotherapy as first-line therapy in advanced breast cancer improved response rate, time to progression, and overall survival.105 Similarly, adjuvant use of trastuzumab significantly improves disease-free and overall survival.106

Despite major advances in the management of HER2-positive breast cancer with trastuzumab, de novo and acquired resistance is common. This has led to a number of alternative strategies to target ErbB2.107 Pertuzumab (Perjeta, 2C4; Genentech, South San Francisco, Calif) is a monoclonal antibody that, like trastuzumab, binds the extracellular domain of ErbB2. However, it binds a different part of the domain that is critical for dimerization of ErbB2 to ErbB3. Preclinical and early clinical trials suggest that pertuzumab is active in trastuzumab-resistant breast cancers and can also enhance trastuzumab efficacy when given in combination. This was recently demonstrated in the Phase III CLEOPATRA trial in women with advanced HER2-positive breast cancer and is under further study in the MARIANNE, NEOSPHERE, TRYPHAENA, and APHINITY trials.

Therapeutic drugs targeting the ErbB family tyrosine kinase domains have been developed.109 Lapatinib (Tykerb, GlaxoSmithKline, London, UK) is a small-molecule reversible inhibitor of both ErbB1 and ErbB2 kinase domains and has been approved for the treatment of HER2+ metastatic breast cancer. Neratinib (HER23C; Pfizer, New York, NY), in contrast to lapatinib, is an irreversible inhibitor of all ErbB kinase domains. Similar to pertuzumab and lapatinib, neratinib has documented activity in trastuzumab-resistant preclinical models and clinical trials and is currently in multiple trials to define its role in the treatment of HER2+ breast cancer.

An alternative and novel approach to strictly targeting HER2 activity is trastuzumab emtansine (T-DM1; Roche, South San Francisco, Calif), a conjugation of an antimicrotubule agent (maytansine) to trastuzumab. Importantly, a comparison of T-DM1 to trastuzumab/docetaxel for first-line treatment of metastatic breast cancer showed both improved response (response rate and investigator-reported progression-free survival) and reduced toxicity for T-DM1.107

Growth factors are major regulators of mammary gland development, but they act via an intricate regulation by the steroid hormones estrogen and progesterone.2 Intriguingly, normal steroid receptor positive mammary epithelial cells do not proliferate in response to steroid hormones, but they send a paracrine signal (most likely IGF and other growth factors) to neighboring cells that then proliferate.110 This paracrine regulation is thought to be critical for the branching morphogenesis of the developing mammary gland. The intricate interaction between steroid hormones and growth factors is likely one of the first pathways to become dysregulated in tumorigenesis, as transcriptomic analysis of early premalignant lesions found elevation of both ER and growth factor (EGF and IGF) signaling.111 Cross-talk between steroid hormones and growth factors is apparent not only in normal mammary development, but also in breast carcinogenesis. For the EGFR/ErbB2 pathway, increased hormone signaling is generally associated with reduced signaling. For example, there is a negative correlation between ErbB2 and ER levels, and ER is a repressor of ErbB2 levels via PAX2.112 In contrast, for the IGF/insulin pathway, ER and PR are both positive regulators, with estrogen in particular upregulating ligand, receptor, and downstream signaling component expression.113 Although IGF-IR and ER are highly correlated in breast tumors, and thus IGF-IR correlates with good prognosis, recent studies examining IGF-IR specifically in TNBC have shown that it correlates with poor outcome and may be a good therapeutic target.114

Experimental evidence from breast cancer cell lines has suggested that a major mechanism of resistance to antihormonal therapy is via upregulation of growth factor receptor pathways.75 However, until recently, results from clinical trials testing this hypothesis have been disappointing, with relatively little benefit from adding anti-EGFR or anti-IGFIR therapies to antihormonal therapy. However, in one promising trial targeting of a signaling molecule mTOR, which is downstream of both IGF-IR and EGFR, showed that the
combination of an mTOR inhibitor (everolimus) and an aromatase inhibitor was superior to the aromatase inhibitor alone in the treatment of hormone-resistant advanced breast cancer in postmenopausal women.115

Angiogenesis

Tumors are generally avascular when they first start to grow; however, as the tumor progresses and increases in size, the distance of cells to blood vessels and nutrients necessitates the new formation of blood vessels (angiogenesis). This angiogenic switch is seen as a critical barrier to tumor growth.116 Preclinical research has identified many critical factors in the angiogenic switch, and blocking this switch with inhibitors of vascular endothelial growth factor (VEGF) has shown benefit in many preclinical models. Clinical testing of VEGF inhibitors (monoclonal antibodies and tyrosine kinase inhibitors) in addition to chemotherapy for women with advanced breast cancer has shown improvements in progression-free survival but little or no effect on overall survival.117 Although it was believed that targeting the host blood supply would circumvent cancer cell intrinsic mechanisms of resistance, resistance to VEGF inhibitors is rapid and via multiple mechanisms.118 Future use of angiogenesis inhibitors in breast cancer will likely require the identification of biomarkers of response to optimize clinical benefit.

Key References


Conclusion and Outlook

Investigating the molecular biology of breast cancer has given tremendous insight into the development and evolution of the disease and highlighted pathways for therapeutic intervention. However, as techniques for interrogating the molecular underpinnings of breast cancer have allowed deeper insight, it is clear that the levels of molecular alteration are much greater than previously anticipated. Indeed, although tumors clearly share certain features (such as ER+ and/or ErbB2+), no two tumors are the same, and the difference in their evolution and expansion provides great challenges for targeted therapies. It is anticipated that the next generation of research will likely tackle two main areas: the heterogeneity of molecular alterations in tumors and the clonal origin of breast cancer. Answers to these two questions are likely to have broad implications for the prevention and treatment of breast cancer.

Acknowledgments

This work was supported in part by research grants from the NIH/National Cancer Institute R01CA94118 (AVL), R01097213 (SO), the Breast Cancer Research Foundation (SO and NED), Susan G. Komen for the Cure (AVL), the Pennsylvania Department of Health (AVL and SO), and the Pennsylvania Breast Cancer Coalition (SO).

All references are available at Expert Consult.