Chapter 3:
Breast Cancer Biology: NCI's Investment and Recent Progress

Given that the two clinical challenges in breast cancer research are to prevent the onset of disease and to effectively treat metastatic disease, the overarching basic biological questions that need to be answered involve understanding the normal and early malignant biology of the mammary gland, and identifying factors responsible for metastatic disease. *Charting the Course: Priorities for Breast Cancer Research*

Basic biology research is fundamentally important for the advancement of many areas of cancer research, as it provides the foundation on which applied studies in early detection, diagnosis, prevention, and treatment are built. In its 1998 report, the Breast Cancer PRG outlined three overarching themes in breast cancer biology research that required specific attention in the coming years: the need to elucidate the normal biology of the mammary gland, the process of malignant transformation in the breast, and mechanisms of breast cancer metastasis. Within these three priority areas, the PRG identified more than 20 subpriorities that could be used to guide the research community in its efforts to understand the basic biology of breast cancer.

In the years since the PRG report, the NCI has generated specific initiatives, Program Announcements and Requests for Applications aimed at addressing the PRG's priorities. Research conducted in response to these efforts, in addition to the broad range of investigator-initiated biology research, has been fruitful, yielding results in breast cancer biology that have fueled progress in other areas of breast cancer research. Significant progress has been made in understanding the biology of mammary stem cells, genetic changes that occur during breast cancer development and metastasis, mechanisms by which tumor-suppressor genes (e.g., BRCA1 and BRCA2) function, and nuclear hormone receptor function.

New mouse models of breast cancer have been generated that closely mimic the different types of breast cancer found in humans. Models such as the BRCA1-null mouse and the Her2/*neu* transgenic mouse have been useful for understanding the biology of estrogen receptor (ER)-negative breast tumors, which are the most difficult to treat.

The information gleaned from these and other biology projects have contributed to progress in the areas of breast cancer prevention, detection, diagnosis, and treatment. For a more comprehensive understanding of this progress, readers are urged to see Chapters 5, 6, and 7 of this report. In addition, basic biology research both influences and is influenced by etiologic research on genetics and biological mechanisms of cancer development and progression. Examples of progress can be found in this chapter and Chapter 4.

NCI's Investment and Response

From FY1998 to 2003, NCI's extramural investment in research on breast cancer biology has increased from $43.2 million to $86 million (Figure 3-1). This increase in funding corresponds to increases in the number of projects that are responsive to PRG priorities in biology. Table 3-1 summarizes the NCI's responsiveness to the three Breast Cancer PRG research priorities for biology and the genetics priorities relevant to biology.[1]

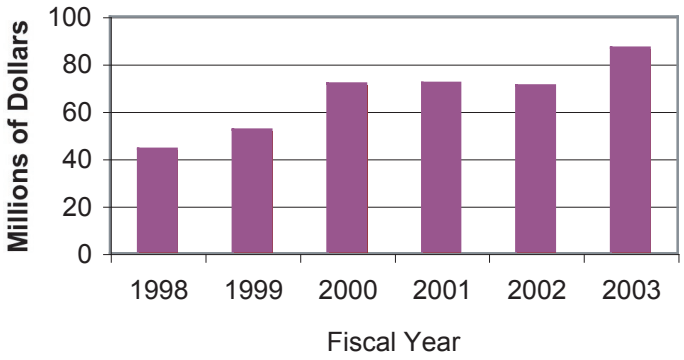


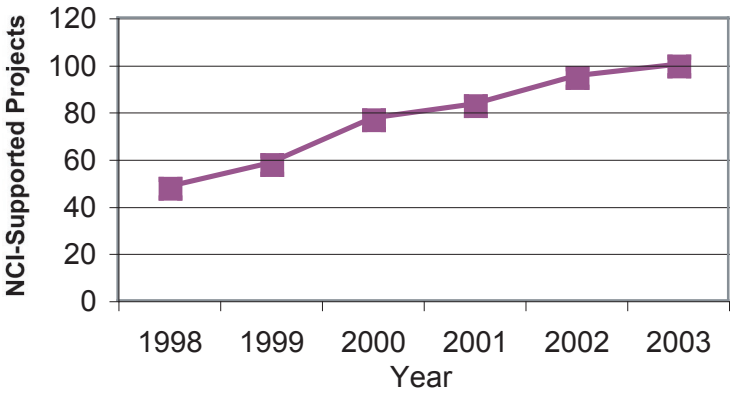
Figure 3-1. NCI's extramural investment in breast cancer biology research: 1998-2003 (in millions of dollars)

1 A project may map to more than one PRG priority and therefore be represented in more than one figure. Projects active in 2003 are listed in Appendix B (Tables B-1 to B-6) by the Principal Investigator's name for each PRG priority.

Table 3-1. NCI Efforts Responsive to PRG Priorities and Opportunities in Biology[a]

PRG Priority:

What are the genetic and biological bases of mammary gland development throughout the life of the organism?[b]



NCI Efforts:

- The original PRG indicated six subpriorities to this priority, which addressed the following topics:
  - The nature of mammary gland stem cells
  - Principal cell types involved in mammary development
  - Control of growth, death, and differentiation during mammary development
  - Critical transcriptional regulatory mechanisms in mammary development, including steroid receptor coactivators and corepressors
  - Principal signaling molecules and pathways in mammary gland development
  - Principal cell cycle checkpoints and their controls in mammary development
- In FY2003, examples of active areas of investigation included vitamin D-mediated apoptosis in mammary cells, prolactin interactions in mammary gland development and tumorigenesis, biology of mammary gland development and tumorigenesis, and *in vivo* cell growth control mediated by C/EBP-β and C/EBP-delta.
- On October 2-3, 2002, NCI sponsored a conference on *Laser Capture Microdissection and Macromolecular Analysis of Normal Development and Pathology*. On July 19-20, 2001, NCI sponsored the *Breast Cancer Think Tank: Mammary Gland Biology Seminar Series* to discuss mammary gland biology with the NCI research community.
- NCI initiatives addressing this priority included the Cancer Genome Anatomy Project (CGAP), Complex Formation in Hormonal Regulation of Gene Expression, Cooperative Human Tissue Network (CHTN), and Mammalian Gene Collection.

PRG Priority:

What are the genetic and epigenetic bases of pathologic lesions that occur during the progression of breast cancer from the earliest hyperplasias to invasive disease?[c] (Biology-B)

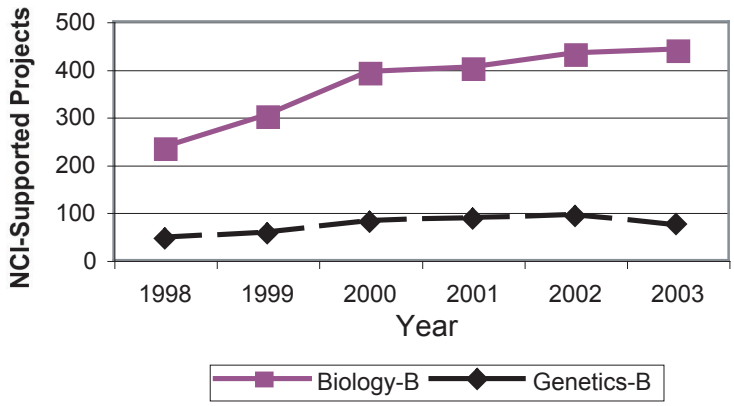
Identify somatic mutations and epigenetic alterations that are due to exogenous factors or to chance. As these are detected, it will be important to know which ones are rate limiting. Once rate-limiting changes are identified, specific pathways altered by these genetic events can provide clues to possible targets for: (1) identifying very small lesions

a. Some of the original PRG priorities are addressed jointly in Table 3-1 because these priorities address partially overlapping issues and they are relevant to many of the same research projects and initiatives.

b. This priority merges the two original PRG priorities: "What are the genetic and biological bases of mammary gland development?" from the Biology section and "Characterize genetic and expression profiles for normal breast epithelium at birth, puberty, adult, pregnancy, lactation, regression, and menopause" from the Genetics section.

c. This priority merges the two original PRG priorities "What are the genetic and epigenetic bases of pathologic lesions that occur during the progression of breast cancer from the earliest hyperplasias to invasive disease; can we develop appropriate diagnostic markers based on these studies?" from the Biology section and "Characterize genetic and expression profiles of breast abnormalities at progressive stages of development from normal to invasive disease" from the Genetics section.

(diagnosis); (2) treatment (by reversing the altered phenotype); (3) identifying tumor cells (to individualize therapy based on tumor genotype); and (4) prevention (by systemic treatment of women before critical changes occur).[d] (Genetics-B)



NCI Efforts:

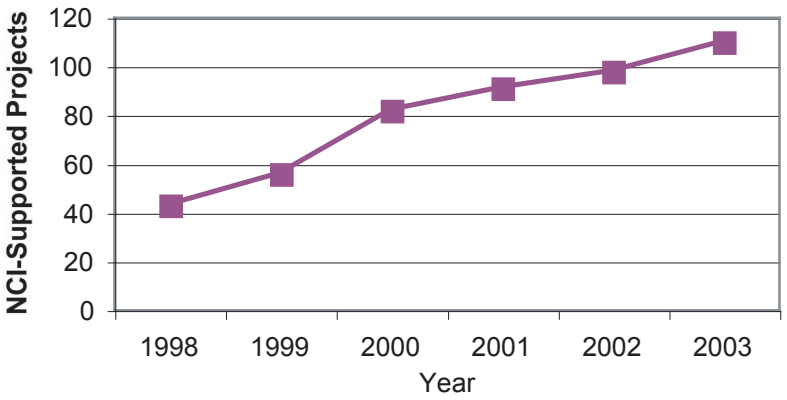
- The original PRG indicated eight subpriorities for the Biology-B priority, which addressed the following topics:
  - Critical signaling pathways in tumor progression
  - The mechanism by which genetic and genomic instabilities are triggered during tumor progression
  - Critical transcriptional regulatory mechanisms in mammary development, including steroid receptor coactivators and corepressors
  - The bases of stem cell-carcinogen interactions
  - Epithelial and stromal cell interactions that are important in tumor cell progression
  - The nature of checkpoint abrogation mechanisms in tumor progression
  - The relative roles of estrogen receptor (ER)-α and ER-β in tumor progression
  - The importance of immune tolerance in tumor progression
- In FY2003, examples of active areas of investigation relevant to the following priorities included:
  - Biology-B: Calmodulin and ER function, role of Her4 as a differentiation factor in breast cancer, role of the tumor suppressor LIBC in inflammatory breast cancer phenotype, the structural basis for ER-β receptor activation, actin-regulated apoptosis in mammary tumorigenesis, molecular alterations in premalignant and *in situ* breast lesions, TGF-β receptor and tumor progression, and identification of allele variant genes that are risk factors for human breast cancer.
  - Genetics-B: Accelerated senescence in tumor cells, a novel HOX gene target in breast cancer, DNA hypermethylation in breast cancer, gene expression signatures of early breast cancer, identification of M5CpG alterations in breast carcinomas, and identification of chromosomal aberrations in human epithelial cancers and hematological malignancies and their respective murine model systems (comparative cancer cytogenetics).
- On October 17-21, 2001, NCI and the American Association for Cancer Research sponsored a special conference, *Cancer and Chromosomal Organization and Epigenetics of Cancer*. On July 19-20, 1999, NCI sponsored the workshop *DES Research Update 1999: Current Knowledge, Future Directions* to address the status of biological, epidemiological, clinical, and education/outreach research on diethylstilbestrol (DES).

NCI initiatives addressing this priority included Applications of Innovative Technologies for the Molecular Analysis of Cancer, Cancer Molecular Analysis Project (CMAP), Cooperative Human Tissue Network (CHTN), Cooperative Breast Cancer Tissue Resource (CBCTR), Director's Challenge: Toward a Molecular Classification of Tumors, and Mouse Models of Human Cancers Consortium.

d. This priority was part of the Genetics section in the original Breast Cancer PRG report.

PRG Priority:

What are the molecular, genetic, and cellular bases of the biological processes involved in metastasis? Can we develop appropriate diagnostic markers based on these studies?

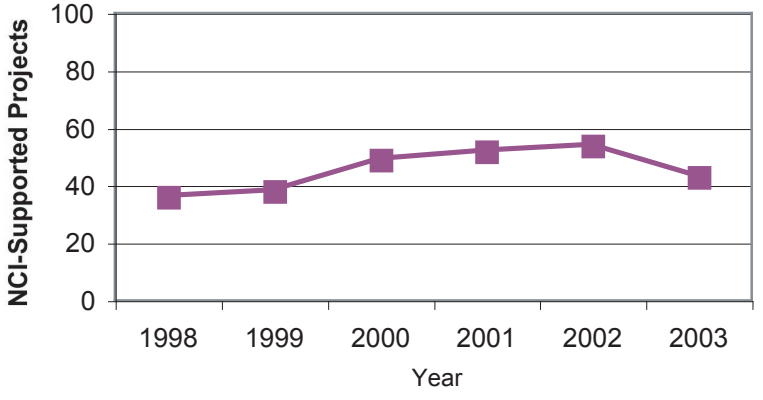


NCI Efforts:

- The original PRG indicated nine subpriorities to this priority, which addressed the following topics:
  - Cell survival pathways operant in metastasis
  - Regulation of tumor angiogenesis
  - Interaction of metastatic cells with bone
  - Control of proteolysis in metastasis
  - Tumor cell motility mechanisms operant in metastasis
  - Importance of epithelial-stromal interactions in metastasis
  - Important signaling pathways in metastasis
  - Cell-cycle checkpoint abrogation mechanisms operant in metastatic cancers that render them more refractory to systemic treatment
  - Aspects of tumor cell physiology of established and metastatic cancers that render them more refractory to systemic treatments
- In FY2003, examples of active areas of investigation included antiestrogenic effects on tumor angiogenesis, a novel function for vascular epithelial growth factor (VEGF) in breast carcinoma survival, biology of VEGFs in the mammary gland, molecular mechanism of tumor-osteoclast interactions, imaging lymphatic clearance in tumor metastasis, effect of obesity on mammary tumorigenesis and metastasis, molecular analysis of breast cancer metastasis to the brain, and molecular biology of the metastatic phenotype.
- On April 25-27, 2002, NCI sponsored the *3rd North American Symposium on Skeletal Complications of Malignancy*.
- NCI initiatives addressing this priority included The Director's Challenge: Toward a Molecular Classification of Tumors, Molecular and Cellular Biology of Metastatic Tumor Cells, Mouse Models of Human Cancers Consortium, and Technologies for Comprehensive, Sensitive, and Quantitative Protein Analysis in Human Tumors.

PRG Priority:

Identify and clone the remaining major predisposing genes.[e]



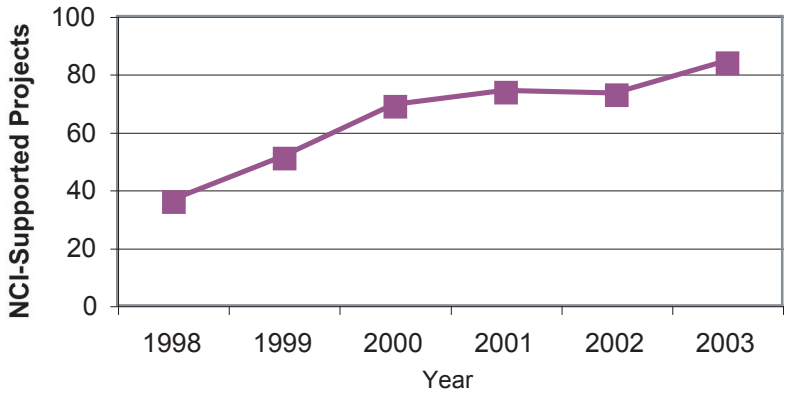
e. This priority was part of the Genetics section in the original Breast Cancer PRG report.

NCI Efforts:

- In FY2003, examples of active areas of investigation included functional analysis of BRCA1, Protein Kinase C as a marker in tamoxifen-resistant breast cancer, the genetics and epigenetics of cancer, identification of allele variant genes that are risk factors for human breast cancer, and the interaction of genes with risk behaviors such as smoking in breast cancer etiology.
- NCI initiatives addressing this priority included the Cancer Genome Anatomy Project (CGAP), Mammalian Gene Collection, and Specimen Resource Locator.

PRG Priority:

Use experimental model systems to determine the effects of mutant genes on the mammary gland, ovary, and endometrium.[f]



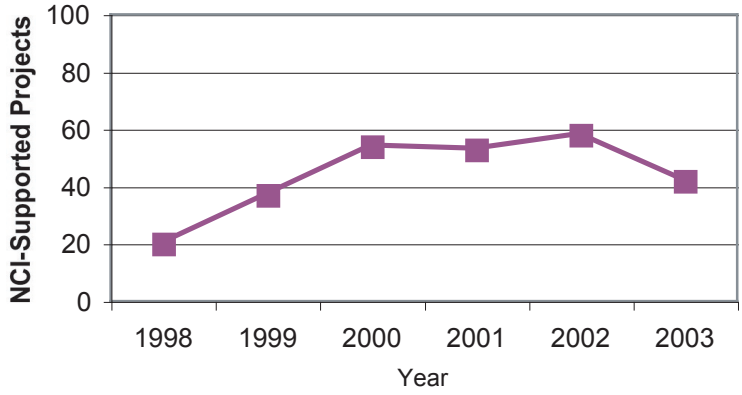
NCI Efforts:

- In FY2003, examples of active areas of investigation included use of an ER-negative transgenic rat mammary model overexpressing *neu* to study chemoprevention; transgenic models for prostate and breast cancer; functional analysis of breast cancer susceptibility genes in mice; identification of chromosomal aberrations in human epithelial cancers, hematological malignancies, and their respective murine model systems; and genetic modifiers of initiation and progression of mammary cancer.
- NCI initiatives addressing this priority included the Molecular and Cellular Biology of Metastatic Tumor Cells, Mouse Models of Human Cancers Consortium, and Stages of Breast Development: Normal to Metastatic Disease.

Additional Breast Cancer Biology Projects:

NCI Efforts:

- In FY2003, examples of active areas of investigation included the role of the prohibitin protein in mediating breast cancer suppression; the Cooperative Breast Cancer Tissue Resource; function of steroid receptors in subcellular compartments; *in vivo* imaging analysis of steroid/nuclear receptor function, tumor oxygenation, vascularization, and radio response; regulation of the sodium/iodide symporter in the breast; and structure of mammary tumor MAT8 and the FXYD proteins.



f. This priority was part of the Genetics section in the original Breast Cancer PRG report and modified from the original "Carry out experimental human genetics in mice, by generating mice with both wild-type and mutant human genes. Determine the effects of these genes on mammary gland, ovary, and endometrium (recognizing mouse-human differences). Determine the effects of mutations against different genetic backgrounds, with the goal of identifying genetic modifiers of mutant alleles."

The NCI has responded to the PRG priorities and recommendations in breast cancer biology by expanding its investment in initiatives relevant to this specific research category. Initiatives that directly impacted breast cancer biology research during the years 1998 to 2003 are described in Table 3-2. In addition to these category-specific initiatives, some initiatives are relevant to many aspects of breast cancer research, including biology. The following list of broadly applicable initiatives is described in detail in Table 2-1:

- Aging Women and Breast Cancer
- Applications of Innovative Technologies for the Molecular Analysis of Cancer
- Bioengineering Research Partnerships
- Breast Cancer Faculty
- Cancer Biomedical Informatics Grid (caBIG)
- Cancer Centers Program
- Cancer Genome Anatomy Project (CGAP)
- Cancer Imaging Program (CIP)
- Cancer Molecular Analysis Project (CMAP)
- Cancer Research Small Grant Program
- Cancer Research Training, Career Development, and Education Opportunities
- Competing Supplements for Organotypic Models of Cancer
- Cooperative Breast Cancer Tissue Resource (CBCTR)
- Cooperative Human Tissue Network (CHTN)
- Director's Challenge: Toward a Molecular Classification of Tumors
- Exploratory Grants for Correlative Laboratory Studies and Clinical Trials
- Flexible System to Advance Innovative Research for Cancer Drug Discovery by Small Businesses (FLAIR)
- Improving DNA, RNA, and Protein Availability in Fixed Tissue
- Insight Awards to Stamp Out Breast Cancer
- Integrating Aging and Cancer Research
- *In Vivo* Cellular and Molecular Imaging Centers (ICMICs)
- Minority Institution/Cancer Center Partnership (MI/CCP)
- Molecular Target Drug Discovery for Cancer
- Mouse Models of Human Cancers Consortium
- NCI Center for Bioinformatics
- Nonmammalian Organisms as Models for Anticancer Drug Discovery
- Shared Pathology Informatics Network (SPIN)
- Shared Resources for Scientists Outside NCI Cancer Centers
- Small Animal Imaging Resource Program (SAIRP)
- Small Grants Program for Cancer Epidemiology

- Specialized Programs of Research Excellence (SPOREs) in Breast Cancer
- Specimen Resource Locator
- Technologies for Comprehensive, Sensitive, and Quantitative Protein Analysis in Human Tumors
- Therapeutic Modulation of Angiogenesis in Disease

Table 3-2. NCI Initiatives Relevant to Breast Cancer Research: Biology[a]

Initiatives Focused on Breast Cancer Research

- Stages of Breast Development: Normal to Metastatic Disease (PA-99-162)
  - Overview: Supports the study of the molecular, cellular, endocrine, and other physiological influences on the development and maturation of the normal mammary gland and alterations involved in early malignant and metastatic breast cancer.
  - Relevant Projects Resulting From This PA: Between 1998 and 2003, 25 projects relevant to breast cancer research were supported through this PA. Specific projects can be found in Appendix B, Tables B1-B5, B7, B9, B11, B14, B19, B30, and B33, by searching for the PA number.

Initiatives With Breast Cancer-Relevant Components

- Bioengineering Nanotechnology Initiative (PA-02-125)
  - Overview: Supports projects on nanotechnology that are potentially useful to biomedicine.
  - Relevant Projects Resulting From This PA: Pending—this PA will remain open until June 3, 2005.
- Complex Formation in Hormonal Regulation of Gene Expression (PA-02-100)
  - Overview: Supports research addressing the role of nuclear hormone receptor complex formation in hormonal regulation of gene expression.
  - Relevant Projects Resulting From This PA: Pending—this PA will remain open until May 1, 2005.
- Mammalian Gene Collection (MGC) (http://mgc.nci.nih.gov/Info/Summary)
  - Overview: A trans-NIH initiative that provides full-length open-reading-frame (FL-ORF) clones for human, mouse, and rat genes.
  - Relevant Results From This Initiative: The MGC has sequenced and verified the complete FL-ORFs for a nonredundant set of 11,666 human genes, 18 of which are breast cancer-specific.
- Molecular and Cellular Biology of Metastatic Tumor Cells (PA-01-020)
  - Objective: Supports the study of molecular and cellular biology of metastatic tumor cells through collaborations that facilitate scientific interchange between investigators with experience in the biology of metastasis and others with more basic scientific disciplines such as molecular biology, cellular biology, andbiochemistry.
  - Relevant Research Projects Resulting From This PA: Between 1998 and 2003, 15 projects relevant to breast cancer research were supported through this PA. Specific projects can be found in Appendix B, Tables B24 and B32, by searching for the current PA number and the previously issued PA numbers (PA-98-029 and PA-93-016).

a. Lists of the projects derived from each initiative can be located in the online Supplement to the Breast Cancer Progress Report: Initiative Database.

- Molecular Interactions Between Tumor Cells and Bone (RFA-CA-03-013)
  - Overview: Supports studies that delineate the role of bone microenvironment on tumor cell survival and colonization. Collaborative interactions among bone biologists, clinical oncologists, and cancer biologists are highly encouraged. These studies contribute toward a better understanding of the molecular events that account for homing of tumor cells to the bone and lead to the generation of novel therapeutic reagents.
  - Relevant Projects Resulting From This RFA: Between 1998 and 2003, three projects relevant to breast cancer research were supported through this RFA. Specific projects can be found in Appendix B, Tables B3 and B33, by searching for the current RFA number.
- Structural Biology of Membrane Proteins (PA-02-108 , PA-02-060)
  - Overview: Supports research leading to the solution of membrane protein structures at atomic resolution, with specific emphasis on membrane proteins and complexes associated with the biology, diagnosis, and treatment of cancer.
  - Relevant Projects Resulting From These PAs: Between 1998 and 2003, one project relevant to breast cancer research was supported through the previously issued PA (PA-99-004): Structure of Mammary Tumor MAT8 and the FXYD Proteins.

Ongoing NCI Research: Recent Progress in Breast Cancer Biology

Mammary Gland Development and Breast Cancer Susceptibility

NCI-supported studies on the basic biology of breast cancer have led to considerable progress toward defining and characterizing the cells and genes that contribute to breast cancer susceptibility, initiation, and establishment. One especially active area focuses on the question of whether breast cancer heterogeneity results from different cells of origin undergoing discrete and different initiating events, alternate pathways of carcinogenesis from a single kind of cancer progenitor, or a combination—i.e., a limited set of progenitors and several common pathways acting in concert—that results in the heterogeneous tumor types that are observed. The answers from studies in a variety of *in vitro* and *in vivo* human and rodent model systems indicate that breast cancer heterogeneity is due to this last mechanism.

Studies of the hematopoietic system and its malignancies clearly demonstrate that there are stem and progenitor cell populations with differing properties that are targets for oncogenesis. Stem cells in adult tissues are able to divide to renew themselves as well as to differentiate into the cell lineages that are needed for normal function of a particular tissue or organ (Weissman et al., 2001). Mammary gland epithelial stem cells possess a high capacity to reproduce themselves, as well as the developmental ability to produce lineage-committed ductal and alveolar cells in the breast, and recent work established that an entire functional mammary gland can be generated from these stem cells (Kordon and Smith, 1998). Mammary stem cells are thought to be essential for the normal growth and differentiation of the mammary gland during puberty, pregnancy, lactation, and involution. Unlike other adult tissues and organs, which have an established morphology, mammary gland architecture undergoes substantial change during these processes.

During the involution process that follows weaning and cessation of lactation, the majority of mammary alveolar cells undergo apoptosis, with the exception of a unique population of epithelial stem cells that appears to undergo expansion at this time. This expanding population of cells increases in size with each subsequent pregnancy. This distinctive population is not present in the nulliparous mammary gland; thus, these cells are hypothesized to contribute to the tumorigenesis resistance mechanism displayed by multiparous women (Wagner et al., 2002). There are several experimental rodent models that point to the *p53* tumor suppressor as a key player in the process of hormone-induced protection (Medina and Kittrell, 2003; Medina, 2004).

Although the concept of breast cancer stem cells is not new, only recently have researchers identified putative stem cells in the mammary gland and demonstrated their role in the development of breast cancer. In a landmark experiment, these rare breast cancer-initiating cells were identified in a population of human mammary tumor cells injected into immunocompromised mice (Al-Hajj et al., 2003). These transformed stem cells are able to give rise to heterogeneous mammary tumors and likely represent the only known population of tumor cells with the capability of initiating tumor growth. These findings have important therapeutic ramifications and shed light on the mechanism by which the induction of premature stem cell senescence reduces the risk of mammary cancer (Boulanger and Smith, 2001).

One particularly important issue for choice of therapy and long-term prognosis is the stem/progenitor cell origin of the breast tumors that are ERα-negative versus ERα-positive; the former are more aggressive, less responsive to therapy, and far more likely to recur. One hypothesis is that the mammary gland self-renewing stem cell is ERα-negative, and in response to differentiation conditions, ERα-positive progenitors arise that continue to self-replicate or differentiate. The ERα-negative stem cell can also give rise via the same or different mutations to ERα-negative cancer stem cell populations with different properties. Subsequent stochastic changes give rise to a number of subsets of breast cancer with differing degrees of aggressiveness and clinical course (Dontu et al., 2004). These hypotheses will require further testing.

The capacity now exists to test these stem/progenitor questions with a variety of model systems. NCI-funded investigators identified a mammary epithelial cell population with several stem/progenitor cell qualities, including expression of stem cell antigen-1 (Sca-1). These markers are useful for both the isolation of functional mammary epithelial stem/progenitor cells and the analysis of tumor etiology and phenotype in genetically engineered mouse models (Welm et al., 2002). Transgenic expression of the Wnt-1 proto-oncogene in the mouse mammary gland causes expansion of epithelial cells that express progenitor cell markers keratin 6 and Sca-1. The resulting tumors express these markers and contain at least two populations of tumor cells—epithelial cells and myoepithelial cells—which implies that they arose by differentiation from an oncogenic progenitor cell. These results suggest that mammary stem and/or progenitor cells may be targets for oncogenesis by Wnt-1. Mammary tumors arising in transgenic mice that express β-catenin and c-Myc, which both encode proteins in the same pathway as Wnt-1, also have a significant proportion of myoepithelial cells and cells expressing keratin 6; however, mammary tumors from transgenic mice that express *neu*, H-*Ras*, or polyoma middle T-antigen contain very few myoepithelial cells or cells expressing markers of progenitor cells. These data suggest that the heterogeneity of tumors in mouse breast cancer models may partially reflect the selection or expansion of different progenitors (Li, Welm, et al., 2003).

Another model that mimics the natural history of a subset of human breast cancer is a *p53*-null mouse that is made in a particular strain background (BALBc). The *p53*-null normal mammary epithelium is as dependent on estrogen and progesterone for growth as *p53*+ epithelium. However, estrogen and progesterone, singly or together, strongly stimulate mammary gland tumorigenesis only in the *p53*-null mouse (Medina et al., 2003). The hormonally transformed ERα-positive cells are serially transplanted into cleared fat pads of *p53*+ mice. The tumors that grow out are often ERα-negative, and serial transplantation of cells from these tumors in ovariectomized *p53*+ mice leads to the development of aggressive tumors, demonstrating that ovarian hormones are no longer required for tumorigenesis. This model should prove valuable for testing chemopreventive agents against either ERα-negative or ERα-positive tumors. This transplantation model is complemented by a *p53*-mutant transgenic mouse model (Lin et al., 2004) in which the activity of *p53* is lost specifically in the mammary gland. The natural history of mammary cancer in this line simulates the transition from ERα-positive to ERα-negative, and both liver and lung metastases are observed. In addition, genetic alterations that accompany human breast cancer progression, such as amplification of c-Myc and activation of Her2/*neu*/*erb*B2, are also reproduced in this model. Effective models of human breast cancer that address defective BRCA1 or BRCA2 can be found in rodents other than mice. Using a very clever technique to screen through the many progeny resulting from germline mutagenesis, a group of investigators was able to identify rat strains that have mutations specifically in these two susceptibility genes (Zan et al., 2003). The rats are being followed for the development of mammary cancer and possible ovarian epithelial cancer.

Due to the multigenic nature of breast cancer, finding all of the genes involved using human population studies is a daunting task. Particularly challenging is the fact that while most susceptibility genes are common, each one has a low penetrance in the general population, which means that many genes probably contribute to susceptibility or resistance. The use of comparative genomics in model systems is increasingly valuable for finding these cooperating alleles. Two studies have used congenic rats to

refine the location of two of the eight susceptibility regions previously identified (Samuelson et al., 2003; Haag et al., 2003). Further refinement is under way to enable isolation of the relevant genes and evaluation of their role in human breast cancer susceptibility. Another research group used a clever strategy that combines the facile genetics of inbred mice and the growing collection of human and mouse SNPs. They substantially narrowed the location of a common susceptibility locus in mice and then searched through SNPs in the human genes in the corresponding human chromosome location to verify the STK15 gene, which encodes the Aurora kinase, as a low-penetrance susceptibility gene in breast cancer. Subsequent studies have shown that this same allelic variant has a role in other common human epithelial cancers (Ewart-Toland et al., 2003). The Aurora kinases normally function to maintain chromosome integrity; if they are overexpressed, they acquire oncogenic properties, causing centrosome amplification, transformation, and aneuploidy. A recent study showed that overexpression of Aurora A kinase is a very early event in rat mammary carcinogenesis (Goepfert et al., 2002); the consistency of the observation regarding centrosome irregularities in a number of human cancers has prompted the development of an Aurora kinase inhibitor that is now in clinical trials.

A number of publications describe innovative new multicell systems to study the interactions of human breast cancer epithelial cells with other cells in the tissue environment. In one case, hydrated collagen gel was used to support studies of the interactions between myoepithelial cells and luminal epithelial cells, and between tumor cells and peritumoral fibroblasts. From these studies, the researchers concluded that myoepithelial cells figure prominently in the maintenance of the correct polarity of the luminal epithelial cells and that direct contact between breast tumor cells and the surrounding fibroblasts causes induction of metalloproteinases and other novel genes (Gudjonsson et al., 2004). Another type of three-dimensional human cell culture system was used to explore the role of a major signal pathway involving PI3-kinase in polarity and proliferation of breast epithelial cells and their malignant counterparts. The study revealed that there are key events in this pathway that have distinct roles for maintaining tissue polarity and that disruption of these events is key to the emergence of malignancy (Liu et al., 2004).

Genetic and Epigenetic Changes in Breast Cancer Development

Breast cancer resembles most other types of cancer in that multiple genetic or epigenetic events must occur in the target cell before it becomes fully malignant. Elucidation of the factors that contribute to mammary tumorigenesis is the common goal of many NCI-supported researchers.

The BRCA1 tumor-suppressor gene is involved in the cellular response to DNA damage and plays a key role in maintaining genome integrity. Half of all inherited forms of breast cancer contain germline mutations in the BRCA1 gene that ablate its function, and an inherited mutation of BRCA1 confers an 85%-90% certainty of female breast cancer by age 70 (Easton et al., 1995). In addition, a significant proportion of high-grade sporadic breast cancers display reduced levels of BRCA1 mRNA and protein, due in part to promoter hypermethylation, a gene-silencing mechanism that is common in human neoplasia (Wilson et al., 1999; Esteller et al., 2000). Researchers studying the structure and function of the BRCA1 protein have recently identified BRCA1-interacting proteins such as BACH1, BARD1, and RAD51 that implicate the tumor-suppressor protein in a variety of cellular activities, including double-strand DNA break repair and transcription of growth inhibitory genes (Cantor et al., 2001; Chen et al., 1998; Boulton et al., 2004).

Amplification or overexpression of the Her2/*neu* receptor tyrosine kinase occurs in about 30% of human breast and ovarian cancers and is associated with a more aggressive course of disease for reasons that may be related to increased proliferation, vessel formation, and/or invasiveness. A breakthrough for the treatment of Her2/*neu*-overexpressing tumors occurred in 1998 with the approval of a new monoclonal antibody-based drug called Herceptin® (trastuzumab). Addition of trastuzumab to the standard chemotherapy regimen yielded higher response rates, longer time to disease progression, and a 20% reduction in the risk of death in women with metastatic breast cancer (Slamon et al., 2001). A recent study demonstrated that younger women with Her2/*neu*-overexpressing breast cancer had an increased risk of developing a secondary tumor in their contralateral breast compared to women whose primary tumors did not overexpress Her2/*neu* (Li, Malone, et al., 2003). Interestingly, these Her2/*neu*-overexpressing cancer cells are more prone to the tumor-promoting effects of ethanol, which was found to increase tumor invasiveness through activation of Her2/*neu* and its downstream signaling components JNK and p38 MAPK (Ma et al., 2003). In laboratory studies, ERα-positive breast cancers that overexpress Her2/*neu* appear to be less responsive to tamoxifen than are

breast cancers with low Her2/*neu* expression. The mechanism behind that observation is in part due to the expression levels of an ERα coactivator, AIB1. If patients' tumors have high levels of both AIB1 and Her2/*neu* expression, their outcome with tamoxifen therapy is very much worse than for either alone (Osborne et al., 2003).

The role of Her2/*neu* in human cancer and the use of Herceptin® prompted the development of a mouse mammary cancer model based on conditional expression of the *neu* oncogene (Moody et al., 2002). When the oncogene is switched on, the mice rapidly develop multiple invasive tumors that readily metastasize to the lungs. When the gene is switched off, which simulates treatment with Herceptin®, the tumors regress both at the primary sites and in the lung. However, the lung metastases eventually recur, and the tumors are no longer dependent on the initiating event: overexpression of *neu*. This is also the experience with patients who are treated with Herceptin®, who initially respond and have improved survival but whose cancer eventually recurs. This model affords the opportunity to study the mechanism of resistance to Herceptin® and to develop more effective therapies for these very aggressive human tumors.

Widespread genetic change is a hallmark of human cancers, and breast cancer is no exception. The application of analysis tools that permit interrogation of gene expression and genome abnormalities is transforming the rate of discovery of genetic alterations. One such tool is chromosomal comparative genomic hybridization (CGH); using this technique, researchers evaluated a set of human breast tumors and found two loci at which abnormalities of the number of copies of genes correlated with poor survival outcome. They were also able to find a relationship between two other loci and the mutational status of *p53* (Jain et al., 2001). Such analyses contribute to gene discovery and the development of prognostic markers. Deletions and amplifications are frequently observed with CGH; amplicons can be sequenced not only to locate genes, but also to study the mechanisms of amplification and their role in cancer progression. One such amplicon at 20q13.2 was comprehensively analyzed by a variety of techniques and found to contain two genes that appear to drive the development of breast tumors (Collins et al., 2001).

Metastasis and Progression

Complications arising from distant metastases are the eventual cause of death in most breast cancer patients whose tumors have spread from their original locations. At diagnosis, approximately 50% of patients with invasive breast cancer have evidence of axillary lymph node involvement, and the presence of cancer at this site is a powerful prognostic indicator for early recurrence and decreased survival (Fisher et al., 1978; Carter et al., 1989). Depending on the estrogen receptor status of the original tumor, further metastases tend to appear in either the bone and reproductive tract (for ER-positive tumors), or viscera (for ER-negative tumors) (Koenders et al., 1991). Bone metastases occur frequently in breast cancer patients, are quite painful, and are usually fatal. The formation of osteolytic lesions is the result of cooperating events between infiltrating tumor cells and resident osteoclasts in the bone. The release of parathyroid hormone-related protein (PTHrP) from tumor cells stimulates the resorption of bone through direct or indirect activation of osteoclasts. Increased osteolysis leads to the release of the growth factor TGF-β near the tumor site, which in turn stimulates further production of PTHrP by the tumor cells. A vicious cycle is thereby propagated in the skeleton, leading to further bone degradation, hypercalcemia, and, eventually, death. NCI-supported research has demonstrated that the downstream effects of TGF-β are mediated by the intracellular signaling molecules p38 MAPK and Smads (Kakonen et al., 2002), and treatment of breast cancer cell lines with a p38 inhibitor was found to prevent TGF-β-induced PTHrP production. Researchers have also demonstrated that lifetime exposure to a soluble TGF-β antagonist protects mice from metastases in a mouse model of metastatic breast cancer (Yang et al., 2002). These findings have important implications for the treatment and prevention of human breast cancer metastases.

Model systems are beginning to reveal some features of the metastatic cascade and the involvement of signal pathways in promoting metastasis. One recent study investigated the role of TGF-β signaling in the progression of metastatic events from invasion of the primary tumor to the establishment of metastatic foci. The investigators did this by engineering a mouse cancer model that can initiate mammary tumors with *neu* and also express either an activated TGF-β type I receptor or a dominant-negative TGF-β type II receptor (Siegel et al., 2003). The activated type I receptor increased tumor latency and enhanced the formation of the lung metastases, while expression of the type II receptor had the opposite effect. Taken together, the results point to a role for TGF-β in the successful extravasation of the breast cancer cells from pulmonary vessels. Another mammary cancer model was used to study the effect of dietary antioxidants on mammary tumor growth and metastasis. These researchers

found that mice fed an antioxidant-depleted diet had more small primary tumors, fewer large primary tumors, and fewer lung metastases compared with mice fed a diet with normal levels of vitamins A and E. This study may aid in understanding why human intervention trials using antioxidant supplements have produced mixed, and sometimes surprising, results (Albright et al., 2004).

Continuing Needs and Evolution

In their 1998 report, PRG members indicated that more research was needed in the area of normal mammary development, particularly with regard to understanding the principal cell types involved in mammary development and their mechanisms of action. Although the number of projects in NCI's Breast Cancer Research Portfolio addressing mammary gland biology has doubled since the release of the 1998 report, there remains a need for more research in this area. This deficiency could be corrected by increasing the funding for new investigators in this field, facilitating the interface between academic investigators and biotech/pharmaceutical companies, facilitating the collection of human breast tissue samples from a variety of developmental stages, and supporting the development and maintenance of new transgenic or knockout mouse models that mimic normal human mammary development.

Progress in breast cancer research would also be accelerated by the integration of biological information across various model systems. Data gleaned from experiments in cell lines, *ex vivo* models, and *in vivo* models could be incorporated into an integrated cancer biology network that uses dynamic computer-based mathematical models, supported by advanced informatics systems, to model the development of cancer. These models could recapitulate the interactive, dynamic, and spatial relationships between molecules in a cell, between cells, between cells and their microenvironment, and between the organism and the macroenvironment during cancer initiation and progression.

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