Honors Thesis: BRCA1 Interactions with BACH1, BARD1, and CHK2: Recent Evidence and Potential Developments in the Diagnosis, Treatment, and Prevention of Human Breast Cancer

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by

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HONORS THESIS: BRCA1 INTERACTIONS WITH BACH1, BARD1, AND CHK2: RECENT EVIDENCE AND POTENTIAL DEVELOPMENTS IN THE DIAGNOSIS, TREATMENT, AND PREVENTION OF HUMAN BREAST CANCER

By Ian Rice

The following thesis focuses on human breast cancer, and specifically the biological underpinnings of most inherited breast cancers. Breast Cancer Associated gene 1 (BRCA1) interactions with three signaling molecules—BRCA1-Associated C-terminal Helicase 1 (BACH1), BRCA1-Associated Ring Domain protein 1 (BARD1), and Checkpoint Kinase 2 (CHK2)—are the focus of the thesis. The BRCA1 gene was discovered in 1994, and in recognition of the ten-year anniversary of its discovery, this thesis surveys 2004 literature on BRCA1, BACH1, BARD1, and CHK2 to identify significant recent contributions to the understanding of their roles and functions. Additionally, this thesis proposes potential developments in the diagnosis, treatment, and prevention of breast cancer based on novel information from the recent literature. BRCA1, BACH1, BARD1, and CHK2 are integrated into DNA repair mechanisms that promote normal cell function and prevent mutations and abnormal growth that may result in cancer. The absence or malfunction of these proteins may result in cancerous cells. Specifically, BACH1 complexes with BRCA1 to unwind damaged DNA, partly comprising a homologous recombination (HR) complex of BRCA1, BRCA2, and RAD51 to repair mutated, deleted, or otherwise damaged nucleotide base pairs. BARD1 is a complex protein that heterodimerizes with BRCA1 to regulate apoptosis and DNA repair functions in BRCA1. CHK2 activates BRCA1 for DNA repair and arrests the cell cycle at the G2 checkpoint. Research of BRCA1 interactions with the aforementioned proteins will hopefully result in molecular cancer therapies that guide oncology in the 21st century.
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INTRODUCTION:

Human Breast Cancer

The following thesis focuses on human breast cancer, and specifically the biological underpinnings of most inherited breast cancers. Human breast cancer is a disease characterized by the excessive proliferation of abnormal breast tissue, generally of ductal or lobular origin (1,2,3). In cancer, abnormal cells eventually gain the function of metastasis, or infiltration, of other bodily tissues. Cancer cells disturb and damage normal cells in various organs, and if left untreated will result eventually in multi-system organ failure and death (1,2,3).

Breast cancer is an uncontrolled growth of abnormal cells in the breast. The two most common sites of such growth are in ductal epithelium or lobular tissue, both of which are involved in synthesis, storage, release, and delivery of milk (1,2,3). Cancer of ductal cell origin is called ductal carcinoma, whereas lobular carcinoma originates in lobules. Ductal carcinomas account for about 80% of all diagnosed breast cancers (1). Lobular carcinomas account for 10-15% of all diagnosed breast cancers (1).

Breast cancer can be either invasive or non-invasive (1,2,3). The non-invasive type remains contained at the site of development (referred to as \textit{in situ}). These cancers can become invasive after progressive loss of regulatory signals and signals involved in cell-cell adhesion and anchorage dependence. Cancer in the breast in not inherently lethal, as breast tissue is not essential for human life. However, when breast cancer becomes invasive, it travels in blood or lymph to distant sites including bone, lung, or
brain tissue. These metastases are lethal, and the potential for breast cancer to metastasize renders it a dangerous disease.

**Breast Cancer and BRCA1**

BRCA1 is an important cellular protein that is often lost or disrupted in many forms of breast cancer. Research on this protein comprises a substantial portion of breast cancer research. Many of the pathways in which BRCA1 is involved have been elucidated by researchers but there is much work to be done in order to completely understand the interconnectedness of all intracellular molecules associated with normal cell function in the breast.

BRCA1 was discovered in 1994 (4). The first mutations involved small insertions or deletions, or nonsense mutations that introduced a stop codon into the transcript causing a nonfunctional protein.

BRCA1 has numerous functions, including DNA repair, cell-cycle checkpoint regulation, ubiquitin ligase activity (which is involved in tagging proteins for degradation), and chromatin remodeling (4). The absence of DNA repair and cell-cycle regulation functions lead to carcinogenesis, thus explaining the relevance of BRCA1 in breast cancer.

The year 2004 marked the ten-year anniversary of the discovery of the BRCA1 gene, and currently breast cancer researchers seem to be standing at the brink of discoveries that will finally permit great strides in improved patient outcome. This thesis concentrates on studies published during 2004 as evidence of the latest elucidations and clarifications of complex cellular signaling networks. The new information in these
findings evokes conjectures and hypotheses of possible future treatments. In short, this thesis will attempt to build the conceptual therapeutic bridges from molecular discovery to effective clinical treatment that breast cancer researchers have thus far been unable to apply to healing medicine.

The interaction of BRCA1 with three signaling molecules—BACH1, BARD1, and CHK2—is the focus of the thesis. A tremendous amount of information about cellular signaling pathways in breast cells is accumulating but has yet to be successfully applied as effective therapy in human breast cancer cases. This thesis speculates as to how current research may become clinically useful in the coming years and decades.

In order to understand this thesis, a background of normal human breast anatomy and physiology is prudent, as is a familiarity with clinical aspects such as the diagnosis, treatment, and prevention of breast cancer. These topics are reviewed first. Then the molecular and cellular biology of breast cancer is introduced with general concepts of cell-cycle regulation, DNA repair, and apoptosis. This culminates in the essence of the thesis: BRCA1 and its interactions with BACH1, BARD1, and CHK2. The thesis concludes by synthesizing these ideas, noting general trends in future breast cancer research, and acknowledging the many challenges facing breast cancer researchers today.
BREAST PHYSIOLOGY AND ANATOMY

The human breasts are mammary glands, a feature shared by all mammals (3). Due to the early weeks of gender ambiguity in development, both men and women have nipples, but otherwise breast development is dictated by Mullerian hormone and female sex hormones in female development (3).

As young women approach puberty, ovarian female sex hormones (estrogen and progesterone mainly, although luteinizing hormone [LH] and follicle-stimulating hormone [FSH] also play a role) develop the rudimentary breast glands into mature adult breasts which are capable of lactation and nourishment of offspring (3).

Simplistically, estrogen elongates the ducts and induces them to create side branches, connecting the 15-20 lobes of milk-producing glands to the nipple for newborn suckling (3). Progesterone promotes development of the milk-producing lobular cells by increasing their size and number. The manifestation of this maturation is breast enlargement.

When menstrual cycles begin, the mammary glands also undergo cyclical changes: in the second half of the cycle (luteal phase), under the increasing influence of progesterone, the glandular tissue grows and sometimes causes ‘lumpiness’ and tenderness — one of many preparations for pregnancy, although a majority of cycles end without fertilization (3). This 'lumpiness' is occasionally mistaken by women as cancerous lumps. Frequent breast self-exams (BSEs) performed throughout the menstrual cycle will familiarize a woman with the normal cyclical changes in the contour and shape of her breasts.
When conception occurs, the increased blood supply to the breasts distends the veins under the skin, comprising the first outward and visible sign of pregnancy. The glandular tissue proliferates, taking the place of connective tissue and fat, and the breasts progressively enlarge. Later in pregnancy, the hormones secreted from the fetal tissue of the placenta act on the glandular cells and on the ducts leading to the nipple to facilitate lactation (3). Increased prolactin during pregnancy would stimulate milk production but placental estrogen antagonizes these effects.

After the birth of the baby, estrogen levels decrease and suppression of lactation ceases, permitting prolactin to stimulate milk production (3). Initial milk production (colostrum) is minimal and watery but is rich in important nutrients. The volume of milk becomes significant approximately three days after parturition, and at this point the breasts are dramatically engorged. Infant suckling stimulates a lactation reflex arc incorporating mechanoreceptors at the nipple, the hypothalamus, and nerves connecting the two. The hypothalamus synthesizes and releases oxytocin, a hormone, into the blood via the posterior pituitary. Oxytocin, when binding ductal cells, will release milk (milk letdown). The entire neuroendocrine reflex requires ten seconds, most of the time taken to release the hormone and circulate it to the target breast tissue. The reflex will continue until the baby stops suckling.
EPIDEMIOLOGY AND CLINICAL ASPECTS OF BREAST CANCER

In 2004 more than 216,000 new cases of breast cancer were diagnosed, and the disease claimed more than 40,100 deaths (1,2,3). The specific cause of breast cancer is unknown and is generally attributed to a wide variety of risk factors, including age, gender, diet, body weight, physical activity level, ethnicity, alcohol intake, smoking, reproductive history, and family history (1). Approximately 5-10% of all breast cancer cases are attributed primarily to this last risk factor, family history. Of these cases, nearly 90-95% are attributed to a mutation in either the BRCA1 or BRCA2 gene (1,3).

After skin cancer, breast cancer is the most common cancer in women, and is the second leading cause of cancer deaths after lung cancer (but breast cancer causes the most deaths in women age 40-55) (2,3). Approximately every three minutes, a woman somewhere in the United States is diagnosed with breast cancer; a woman dies of breast cancer every 12 minutes (2). A woman's risk of developing breast cancer increases with age; more than three out of four breast cancer cases occur in women over age 50 (1,2). Although breast cancer is much more common in women than in men, about 1,400 new cases of breast cancer are diagnosed each year in men in the United States, with 470 deaths (1).

Common risk factors include a family history of breast cancer; never having been pregnant, or having a first pregnancy after age 30; being overweight, especially after menopause; drinking alcohol (cancer risk doubles with three or more drinks per day); and living a sedentary lifestyle with little regular exercise (1,2,3).
Today the lifetime probability of a woman developing breast cancer is 1 in 8 (12.5%), a significant increase from the 5% probability for women in 1940 (1,3). This difference is likely due to environmental influences and lifestyle choices. This is supported by the fact that 70% of breast cancers occur in women with no known risk factors (1). Nonetheless, approximately 5-10% of all breast cancers are caused by hereditary deletions and mutations in important regulatory genes (ie. BRCA1 and BRCA2) (1,3). Additionally, having a first degree relative (mother, daughter, sister) with breast cancer doubles the risk of breast cancer, and having two first degree relatives increases the risk 5-fold (1,2,3). Thus, breast cancer is a disease resulting from a complex interaction between genetics and environment.

**Symptoms**

Common symptoms of breast cancer include a lump in the breast or under the arm; a clear or bloody discharge from the nipple; persistent crusting or scaling of the nipple; inverted nipples; redness or edema of the breast; dimpling on the breast (resembling the texture of an orange); a change in the contour of the breasts; or a sore or ulcer on the breast that does not heal (2,3).

**Diagnosis**

The most important diagnostic tool in fighting breast cancer is the most rudimentary: the breast self examination, or BSE (1,2,3). Women are encouraged to perform BSEs on a monthly basis regardless of age or risk. In the formal clinical setting, a physician will perform a physical examination (monitoring for the above symptoms) and address patient history, identifying potential risk factors. Based on this information, a
physician may order a screening mammogram (essentially an X-ray of the flattened breast tissue), an MRI, an ultrasound, or ductal lavage, which can evaluate ductal cells (1,2,3).

Mammography is much less effective in younger women because breast tissue is more dense than in older women and because breast cancer in younger women is generally more aggressive in its progression, meaning the tumors grow at a faster rate (2,3). Recent studies indicate that ultrasound and MRI examinations are better suited for younger patients, although either procedure may also be used for older women (2,3). Ultrasound examination is most commonly utilized to determine whether a lump is a solid tumor or a fluid-filled, noncancerous cyst. Solid lumps are usually evaluated further.

If a lump is detected by physical palpation or identified by screening and deemed to be potentially cancerous, a biopsy will be performed to evaluate the nature of the lump. Several types of breast biopsy are currently available (including fine-needle biopsy, core biopsy, stereotactic needle biopsy, sentinel node biopsy, and surgical biopsy, which involves the removal of all or part of the breast lump) (3). The biopsies vary from being performed in an exam room (needle biopsy) to the operating room if a large sample is extracted (surgical). A pathologist then evaluates the tissue sample and further classifies its nature.

The biopsy will confirm whether the lump is breast cancer. Depending on the specific type of biopsy, and whether neighboring lymph nodes were also removed and examined, the biopsy report also may clarify the extent of cancer spread, whether the
tumor cells are estrogen-receptor positive or negative, and whether there are too many
copies of the Human Epidermal growth factor Receptor-2 (HER-2) gene (discussed
further in the next section) in the cancer cells, for example (2,3). These factors partly
determine the type of recommended treatment.

Malignant breast cancer diagnoses generally fall into one of these five
descriptions: ductal carcinoma, lobular carcinoma, medullary/mucinous/tubular
carcinomas, Paget’s disease, and inflammatory carcinoma (3). Ductal carcinoma in situ
(DCIS) occurs when cancer cells fill the ducts but have not yet spread through the walls
into fatty tissue. Nearly all women diagnosed at this early stage can be cured. Without
treatment, about 25 percent of DCIS cases will lead to invasive breast cancer within 10
years. Invasive ductal carcinoma can break through the duct wall and invade the breast's
fatty tissue, then metastasize to other parts of the body through the bloodstream or
lymphatic system. Prognosis for patients becomes significantly worse when ductal
carcinoma reaches the invasive stage.

Lobular carcinoma in situ (LCIS) is less common and less of a threat than DCIS.
It develops in the breast's milk-producing lobules. LCIS does not require treatment, but it
does increase a woman's risk of developing breast cancer. If LCIS continues to progress,
invasive lobular carcinoma may develop and can spread to other places in the body.

Medullary, mucinous and tubular carcinomas are three slow-growing types of
breast cancer (3). Together they represent about 10 percent of all breast cancers.
Paget's disease represents about one percent of breast cancers (3). It starts in the milk ducts of the nipple and can spread to the areola. Women who get Paget's disease usually have a history of nipple crusting, scaling, itching or inflammation.

Inflammatory carcinoma accounts for about one percent of all cases (3). Of all breast cancers, inflammatory carcinoma is the most aggressive and most difficult to treat, because it spreads so quickly. Prognosis is correspondingly poorer than other breast cancers.

In addition to the categorical diagnosis of the condition, a quantitative assessment is provided by the physician in the form of staging, and the tumor will be allocated to a stage between zero and four (1,2,3). Staging reflects the extent of the spread of the tumor, whereas grading reflects the tumor and cellular morphology, such as the degree of differentiation, the regularity of tumor borders, and the aggressiveness of the cells.

Fortunately, 80% of all breast lumps are benign (2,3). Benign breast lumps usually have smooth edges and can be moved slightly. They are often bilateral (found in both breasts). Benign breast lumps arise from various common causes including normal changes in breast tissue, breast infection or trauma, and side effects of certain medication. Benign tumors may progress into cancer if proper and prompt treatment is not taken.

Benign tumors can be classified into one of the following categories (2,3):

Fibroadenomas are the most common benign tumors found in the female breast (2,3). They are solid, round, rubbery lumps that move freely in the breast when pushed upon and are usually painless. Fibroadenomas are the result of excess formation of
lobules (milk-producing glands) and surrounding breast tissue. They occur most often between the ages of 20 and 30 and are more common in African-American women.

Women with fibrocystic breast disease typically experience lumps in both breasts that increase in size and tenderness shortly prior to menstrual bleeding. Nipple discharge is not uncommon. The lumps are ducts and surrounding tissues that have expanded to form cysts. The cysts rapidly enlarge in response to increased estrogen and progesterone near menstruation and may be hard or rubbery upon palpation. Fibrocystic disease is the most common form of benign breast lesions in women ages 35 to 50. Postmenopausal women are less likely to have these types of breast changes because hormone stimulation wanes considerably.

Intraductal papillomas are small growths in the ductal epithelium that occur near the nipple and resemble warts on physical examination (3). Intraductal papillomas most commonly afflict women 45 to 50 years of age and can produce bleeding from the nipple.

Simple cysts are fluid-filled sacs that usually present bilaterally (2,3). They can be single or multiple and can vary in size. Tenderness and lump size often vary cyclically with menstruation.

Traumatic fat necrosis results from acute trauma to the breast and causes fatty, round, firm, and painless lumps (1,2,3).

**Treatment**

The three major goals of all cancer therapies are as follows, in order of importance: eliminate or rid the body of cancer; ensure that the cancer will not return; minimize patient discomfort throughout treatment.
Treatment for breast cancer almost always includes surgery. Generally, the two surgical options are removing the entire affected breast (mastectomy) or removing only the malignant lump and a margin of healthy tissue around it (lumpectomy) (2,3). There are four mastectomy procedures: total mastectomy, radical mastectomy, modified radical mastectomy, and segmental mastectomy (also known as partial mastectomy or Quadrantectomy) (3). Surgery may be followed by radiation therapy, and sometimes chemotherapy, to destroy any remaining cancer cells. Radiation therapy decreases the probability of cancer recurrence by approximately 25 percent (1,2,3).

The need for chemotherapy depends on the extent of spreading (2,3). In some cases, chemotherapy will be recommended before surgery to shrink a large tumor, facilitating easier removal. Chemotherapy is almost always necessary if cancer recurs and is administered in cycles for three to six months. A form of chemotherapy called hormonal chemotherapy usually is recommended when the pathology report shows that the cancer is estrogen-receptor positive (3). In hormonal chemotherapy, the drug tamoxifen (Nolvadex) is taken daily, by mouth, for two to five years. Tamoxifen blocks estrogen from breast cancer cells that are estrogen-receptor positive, which may reduce the cancer recurrence rate by up to 30 percent (tamoxifen is discussed further in the Prevention section) (3). Tamoxifen is generally only beneficial for estrogen-receptor positive breast cancers, as this type of breast cancer is dependent on estrogen as a growth signal. Tamoxifen is generally not effective against estrogen-receptor negative breast cancers, which proliferate regardless of estrogen presence. Chemotherapy is infamous
for its unpleasant side effects (including but not limited to): nausea and vomiting, loss of appetite, hair loss, mouth sores, bruising, bleeding, and fatigue (1,2,3).

In the past, DCIS was treated as if it were invasive breast cancer, but now it appears that less aggressive treatments may be equally effective (3). Though mastectomy is still performed for DCIS, lumpectomy with radiation also is a common and less severe alternative. In some women, lumpectomy without radiation also may be effective. Because LCIS develops slowly, typically no treatment is initially required, but women with this condition are often recommended regular mammograms and breast exams by a physician to monitor for potential migration of the carcinoma.

A recent addition to conventional therapies is biological or molecular targeted therapy (2,3,5). The most significant biological treatment to date is Herceptin (trastuzumab), a chemotherapeutic agent and monoclonal antibody that blocks the human epidermal growth factor receptor 2 (HER-2) protein (2,3). When HER-2 is bound by epidermal growth factor, breast cells initiate signaling cascades that promote cell growth. In the case of cancer cells, HER-2 activation promotes cell growth in abnormal, cancerous cells, which is undesirable for a patient with breast cancer. Additionally, Herceptin attracts Natural Killer (NK) cells that initiate an inflammatory apoptotic pathway. Thus, in addition to blocking growth signals via blocking HER-2, Herceptin also promotes cell death on cancerous cells, representing a dual mechanism for controlling and eliminating cancerous breast cells. Approximately 25% to 30% of breast cancers overexpress HER-2, and only these cancers are significantly affected by
Herceptin. Herceptin has many advantages over chemotherapy, most notably the absence of many side effects associated with chemotherapy.

**Prevention**

Although there is no proven method for completely preventing breast cancer, the following precautions can greatly reduce the risk of developing the disease: not smoking, maintaining a healthy weight, exercising regularly, eating a diet high in cruciferous vegetables, performing a BSE every month, seeking a clinical breast examination by a physician every three years under age forty and annually after age forty, having regular mammograms, and limiting alcohol consumption (1,2,3). A folate supplement (800mcg daily or with any alcoholic drink) may negate some of the increased risk caused by alcohol consumption (3).

Annual screening mammography allows breast cancer to be detected at an earlier stage; most experts agree that women 50 years and older should have annual mammograms (1,2,3). The American Cancer Society and the National Cancer Institute recommend screening beginning at age forty (1).

Studies have investigated the utility of preventive tamoxifen administration in high-risk patients (2,3). Tamoxifen is an estrogen antagonist, thereby eliminating a significant external proliferatory signal to breast cells. Although originally used as long-term post-surgical treatment in breast cancer remission, doses of tamoxifen have been effective in preventing estrogen-receptor positive breast cancer. As a side benefit, tamoxifen also reduces the risk of a heart attack by reducing the level of inflammatory agents in the blood (these agents could cause bursting and release of arterial plaque
deposits, which in turn may block a cardiac artery and lead to myocardial infarction, a life-threatening condition). However, tamoxifen is not an effective method of breast cancer prevention for everyone. As previously mentioned, tamoxifen is not an effective treatment against estrogen-receptor negative breast cancer, and expectedly is also not a useful drug for prevention of estrogen-receptor negative breast cancer. Additionally, preventive use of tamoxifen is a difficult decision due to numerous side effects, including the discomfort of hot flashes, nausea, light-headedness, loss of hair, rashes, visual disturbances, contra-indications with anticoagulants, and an increased risk of both endometrial cancer and venous thrombosis (2,3).

Alternatives to tamoxifen are being currently developed and tested in clinical trials, including Arimidex and Femara (produced by Novartis), both in the new family of aromatase inhibitor drugs (2,3). By inhibiting aromatase, these drugs prevent the conversion of testosterone to estrogen in the ovaries, thus starving breast tumors of the growth signal it needs for proliferation and metastasis. Like tamoxifen, these drugs do not come without disadvantages and limitations, including decreased bone density and increased osteoporosis risk.

Arimidex is an aromatase inhibitor drug from AstraZeneca PLC (2,3). Arimidex does not kill the tumor cells but does promote a quiescent state, and thus may be useful as a supplement to conventional therapy. The new five-year results of the recently published study are promising (2,3). Women on Arimidex were 13 percent more likely to be alive and cancer-free than those on tamoxifen, the current standard for long-term breast cancer treatment following more aggressive therapies including surgery, radiation
therapy, and chemotherapy. Tamoxifen is an estrogen receptor blocker, and thus Arimidex aims to achieve a similar goal but from a different, apparently more effective, perspective. Arimidex also cut the risk of cancer developing in the other breast by 42 percent over tamoxifen, and of spreading to other places in the body by 14 percent. Researchers do not know why aromatase inhibition seems more effective than estrogen receptor blockage.

Unfortunately Arimidex will not be effective for everyone (2,3). Estrogen-receptor negative breast cancers, which do not need estrogen to grow uncontrollably, will likely not be halted or even slowed by Arimidex. Also, premenopausal women can not take Arimidex due to the severe side effects of disturbing the body’s menstrual-ovarian cycle and hormonal balance. There are also concerns that Arimidex will exacerbate problems like osteoporosis in older patients. Overall, Arimidex appears to be another tool in treating breast cancer and will be effective in some patient populations.
BIOLOGICAL ASPECTS OF BREAST CANCER

Tumor Suppressors: BRCA1

BRCA1 is generally classified as a tumor suppressor gene, along with other ubiquitous and notable genes such as p53, Rb, p21, and others (4,5,6,7,8,9,10). The functions of tumor suppressor genes and the proteins they encode are analogous to the brakes in an automobile. Tumor suppressors (TS) engage in many functions, and many TS can identify DNA errors and perform one of two functions: repair the DNA, or initiate a cascade resulting in programmed cell death, known as apoptosis (4,5,6,11).

Either way many TS stop the cell cycle and some TS initiate one of the two responses. These functions are analogous to a computer anti-virus program, which initially attempts to clean an infected file and if unsuccessful will resort to quarantining and deleting a file. TS can do this in the cell by initially preferring DNA repair and resorting to apoptosis as necessary (4,11). Loss of TS function, either by a faulty gene or protein, will result in the accumulation of DNA mutations and errors and eventually unchecked cellular growth (4,11). Abnormal, incessantly dividing cells result.

DNA repair, the preferred option versus apoptosis in the cell, may occur in one of two ways: homologous recombination (HR), or non-homologous end-joining (NHEJ) (4,6). HR occurs when the DNA repair complex (discussed later) is intact and fully functional. Complimentary DNA strands are matched and bound together, and matching bases are added to fill in gaps. Because the DNA is recombined in a way that matches bases with their appropriate complements, it is referred to as ‘homologous.’ In contrast, NHEJ is an inferior and typically error-prone DNA repair mechanism because it can
result in chromosomal rearrangements that cause instability and are conducive to carcinogenesis (4,6). In NHEJ, bases are randomly and haphazardly placed in gaps and at the ‘sticky ends’ of DNA. This provides a moderately stable organization that allows the cell cycle (including mitosis) to continue, but inevitably errors accumulate over successive cell divisions.

**The Roles of BRCA1**

The BRCA1 protein plays a central role in the DNA repair pathway, and for this reason the loss or mutation of the BRCA1 gene is strongly associated with hereditary breast cancer (4,5,6,7,8,9,10). Specifically, BRCA1 plays a role in HR, where it associates with RAD51 at the site of DNA damage (5). It is unclear how BRCA1 operates in the repair mechanism, but when it associates with RAD51 it becomes phosphorylated and active. If the cell has low levels of BRCA1 or mutated forms of the protein, DNA repair will not occur and errors may accumulate from mutagenic factors, such as ionizing radiation that causes double strand breaks (DSBs) in DNA (5,6,7).

BRCA1 repairs DSBs via the HR mechanism (5). However, if BRCA1 is expressed at an insufficient level or is mutated in the cell, the default DNA repair mechanism is NHEJ, resulting in more problems and instability in the long run (4,6). In contrast to HR, NHEJ is typically an error-prone process in which nucleotide alterations are tolerated at the sites of rejoining (6).

In addition to HR, BRCA1 is necessary for cellular processes ranging from cell cycle checkpoint control, DNA repair, and regulation of transcription to protein ubiquitination, apoptosis, and chromatin remodeling (4,5,6,7,8). BRCA1’s dual role of
cell-cycle arrest in conjunction with DNA repair is important because continued replication of error-ridden DNA is wasteful and harmful to the cell until the DNA is repaired (4,5,6). The actual mechanism of cell cycle arrest is still being studied.

Of the many types of DNA damage, DNA DSBs represent a particularly dangerous form of damage (4,6,12). If not properly repaired, a DSB causes genetic changes and/or cell death (4). DSBs can arise spontaneously or may be induced by exogenous DNA damaging agents (4). DSBs, like other DNA damage, is repaired by either HR or NHEJ. HR requires an undamaged template molecule that contains a homologous DNA sequence ordinarily on a sister chromatid or a homologous chromosome. HR is mediated through multiple proteins, including the Rad51/Rad52 recombinases, BRCA1, and BRCA2 (4,6). BRCA1 colocalizes with BRCA2 and Rad51 and forms ionizing radiation (IR)-induced subnuclear foci (IRIF) containing the Rad51 protein (4,8). Thus, because DSBs are so damaging to a cell, and because BRCA1 mediates the HR that repairs DSBs, BRCA1 is vital to healthy cell growth and division.

The importance of BRCA1-mediated HR versus NHEJ can not be overstated. BACH1, BARD1, and CHK2 all facilitate BRCA1-mediated HR in some capacity, and it is for this reason that they have recently garnered attention from researchers. The success of potential clinical applications will hinge on the ability to halt cellular activity in cancerous cells and consistently promote the preferred HR rather than NHEJ.
BRCA1 & BACH1 INTERACTIONS

BRCA1 associated C-terminal helicase 1 (BACH1) is a helicase that complexes with BRCA1 in BRCA1’s C-terminal domain (the BRCT motifs) to unwind damaged DNA, partly comprising an HR complex of BRCA1, BRCA2, and RAD51 to repair mutated, deleted, or otherwise damaged nucleotide base pairs (4,6,13,14,15) (Figure 1). BACH1 serves with these other HR complex proteins in a caretaker-type tumor suppression role, meaning that it maintains normal cell function by fixing errors and ensuring normal, controlled growth. As a helicase, the primary role for BACH1 is to unwind the DNA duplex for BRCA1, allowing BRCA1 to initiate double-stranded break repair (DSBR) (4,13,14,15).

The first suggestion that BACH1 might be critical to BRCA1 tumor suppression function was the observation that tumor-predisposing missense and deletion mutations in the BRCA1 BRCT domain, all of which render BRCA1 defective in its HR function, also disrupt BACH1 binding to BRCA1 (4,12,13,14,15).

Recently, it was shown that the interaction between BRCA1 and BACH1 depends on the phosphorylation status of BACH1 and that this phosphorylation-dependent interaction is required for DNA damage-induced checkpoint control during the G2/M phase of the cell cycle (13,14,15). Thus, although the purpose of BACH1 is as a helicase to facilitate DSBR, its associated consequence of checkpoint control is also important for normal cell growth (by unwinding the DNA, BACH1 forces a pause in the cell cycle, and this is the associated consequence).
More recently, it was demonstrated that BACH1 is a DNA-dependent ATPase and that its ATP-dependent DNA helicase translocates in a 5’-to-3’ direction (13,14,15). BACH1 can unwind not only DNA:DNA substrates but also RNA:DNA hybrid substrates (14). However, BACH1 can not unwind DNA strands of varying length with equal efficacy, as it is able to completely unwind short strands of DNA and only partially unwind longer strands (13,14,15). This supports its role as a DNA helicase because helicases only open a small stretch of DNA at a given time for repair—in fact, a helicase that opens long stretches of DNA at a time would create instability in the repair complex, and the DSBR would be slower and less thorough. DNA is unstable in an unwound, single stranded state, so BACH1’s proficiency in unwinding only short segments of DNA reflects its optimal capacity for DSBR.

Experiments investigating the affinity of BACH1 to full-length BRCA1 and a truncated BRCA1 containing only the BRCT repeats revealed that BACH1 binds each with equal frequency and strength (13,14,15). This implies both that the non-BRCT sections of BRCA1 do not contain any necessary binding sequences for BACH1 and also that the conformation of the full-length BRCA1 protein does not sterically hinder or interfere with BACH1 binding.

Furthermore, the binding domains between BACH1 and BRCA1 are becoming increasingly well-defined—recent mapping results indicate that BACH1 residues 979-1006 are sufficient for BRCA1 binding in vivo (14). These findings are consistent with recent data indicating that the BRCA1–BACH1 interaction is mediated by a segment of this sequence that requires phosphorylation of serine 990 (14,15).
BACH1 is intimately involved in DNA metabolism by virtue of its role as a DNA-dependent ATPase and DNA-dependent helicase (13,14). Loss of BACH1’s role in these activities has been correlated with breast cancer (13,14).

Clinical confirmation of this came from a study of sixty-five women with early-onset breast cancers (9,13). They were screened for germline BACH1 mutations—researchers detected two distinct heterozygous missense alterations (P47A and M299I) affecting the BACH1 helicase domain. These alterations were absent among 200 healthy controls and, therefore, are unlikely to represent common, coincidental polymorphisms. The P47A mutation represents a base pair mutation that causes the integration of an alanine amino acid into the protein at the 47th amino acid location, whereas in the normal BACH1 protein there should be a proline amino acid. In related fashion, the M299I mutation represents a base pair mutation that causes the integration of an isoleucine amino acid into the protein at the 299th amino acid location, whereas in the normal BACH1 protein there should be a methionine amino acid. The mutations may seem insignificant in a protein consisting of hundreds of amino acids, but the 47th and 299th amino acid locations are particularly important for normal BACH1 function. The P47A substitution occurred in a family with a strong history of breast and ovarian cancers and is associated with BACH1 protein instability. The proline residue seems to be a critical residue for DNA repair activity, which may explain the correlation. Thus, even though the 47th and 299th amino acids of BACH1 are not part of the 979-1006 binding domain for BRCA1, they are still necessary for the helicase function and hence BACH1’s purpose in the HR complex.
Another experiment determined that a mutation of the 299th amino acid in BACH1, from a methionine to an isoleucine, generated an increase in ATPase activity (14). The reason for the increase is uncertain, but nonetheless this position on BACH1 influences ATPase activity. Contrary to expectations, the elevated ATPase activity did not result in increased helicase activity, even though the helicase activity is ATP-dependent (14). Compared to wild-type BACH1, the mutated BACH1 could not effectively unwind longer DNA strands, although it was still able to unwind short strands (14). This unexpected result from the M299I mutation and consequent increased ATPase activity may be explained by many possibilities, including one of the following: first, the increased ATPase activity from the M299I mutation might result in an abnormal helicase that unwinds short DNA strands rapidly but cannot coordinate its activity on longer DNA strands. In particular, the M299I protein might carry out futile ATP hydrolysis that is not coupled to translocation along the DNA strand. Another possibility is that the M299I mutation perturbs the ability of BACH1 to form active, higher-order complexes.

Support for the second possibility comes in the recent isolation of BACH1 in two forms: as a megadalton size complex that contains BRCA1 and BARD1; and as a 500-kDa complex that appears to only contain BACH1 (14). Whether the M299I mutation prevents proper assembly of active BACH1 must be investigated in future research. Nevertheless, the larger complex suggests a unified purpose for BRCA1, BACH1, and BARD1 in DSBR (14). BARD1 is discussed in detail in the next section, but the connection between BRCA1 and BACH1 is becoming increasingly well-established—BRCA1 directs HR, and BACH1 is the helicase that opens the double-stranded DNA and
provides access to the DNA for BRCA1. This finding—that BACH1 exists in equilibrium between an independent state and in a DSBR complex—comprises one of the surprising findings of 2004 regarding BACH1 and BRCA1 interaction because previous research had merely delegated BACH1 to either a complex or independent state, incapable of conversion. In fact, there is a dynamic equilibrium of BACH1’s state and the ratio of complex to independent BACH1 can change to meet the needs of the cell throughout the cell cycle. Thus BACH1 represents a dynamic, not static, protein.

Potential diagnostic methods may include an analysis of BACH1 levels in breast cancer cells and the state of BACH1—whether it predominantly exists in the large complex or in its independent state. Deficiencies in the amount of BACH1 complexes may indicate a problem in binding with BRCA1 and the other DSBR components. Also, testing for the two common mutations (P47A and M299I) may provide physicians with another dimension in assessing a patient’s risk profile. Exogenous BACH1 supplementation may become a beneficial adjuvant therapy if it is determined that endogenous BACH1 is normal and functional but underexpressed, or is expressed in a nonfunctional state. Admittedly, this solution is theoretically appealing but is substantially limited in reality due to the fact that cells typically do not take up exogenous proteins, and packaging the protein in a vector that can carry it across the plasma membrane has proven elusive. Typically attempts at constructing a vector have produced results lacked efficacy or sufficient specificity, since typically a treatment is intended only for the cancerous cells and is not safe or beneficial if exposed to all cells in the body. This limitation in exogenous supplementation will also apply to proposed
therapies for BARD1 and CHK2, which are also proteins that will typically not cross the membrane. Surmounting the issue of exogenous supplementation will require much further study and is a major obstacle in successfully applying BRCA1, BACH1, BARD1, and CHK2 research to clinical therapies.
BRCA1 & BARD1 INTERACTIONS

BRCA1 associated ring domain 1 (BARD1) is a complex protein that heterodimerizes with BRCA1 in the N-terminus RING domain of BRCA1 to regulate the dynamic equilibrium between BRCA1 role in apoptosis and DNA repair (4,7,16,17,18,19,20,21) (Figure 1). BARD1, bound to BRCA1, retains BRCA1 in the nucleus by binding and blocking the nuclear export sequence on BRCA1 (4,17,18,19,20,21) (Figure 1). This nuclear retention promotes nuclear DNA repair by BRCA1 and does not permit BRCA1 to leave the nucleus and initiate apoptosis by a p53-independent mechanism (8,17,18,19,21). The specific dynamics and contributing factors in the mechanism are currently unknown. Conversely, if unbound by BRCA1, BARD1 can also migrate from the nucleus to the cytoplasm and initiate apoptosis by a p53-dependent mechanism (16,17,18,19,21). Other than the dependence of p53, the specific dynamics and contributing factors of this mechanism are also unknown. Thus, balance of BARD1 and BRCA1 are crucial in determining cell fate. If BARD1 and BRCA1 are in relatively even nuclear concentrations, the equilibrium favors DNA repair. On the other hand, if either BRCA1 or BARD1 is significantly higher in concentration than the other in the nucleus, then the excess protein may escape to the cytoplasm and initiate apoptosis. These relationships are depicted in Figure 2.

Recent studies have determined that cytoplasmic BRCA1 induces apoptosis but can not when bound to BARD1 in the nucleus because it is not in the cytoplasm (8,16,17,18,19,21) (Figure 2). In other words, BARD1 inhibits cytoplasmic BRCA1-dependent apoptosis. In contrast to apoptosis in the cytoplasm, BRCA1 induces cell-
cycle arrest in the nucleus. Unlike BARD1’s inhibition of cytoplasmic BRCA1-mediated apoptosis, BARD1 dimerization with BRCA1 does not block nuclear BRCA1-dependent cell cycle arrest (16,17,18,19). Furthermore, BARD1 and BRCA1 are both phosphorylated, bound, and active as part of the HR complex for DSBR. Thus BRCA1-BARD1 heterodimerization is conducive to cell preservation and repair but not to apoptosis, seemingly ensuring this is the last option for the cell.

Recent studies have demonstrated that BRCA1-BARD1 heterodimerization domains are necessary on both proteins for proper dimerization (16,17,18,19,21). In one experiment, researchers deleted most of the dimerization domain in BRCA1 by deleting the first seventy residues of the protein (18). They found that this mutated protein carries out apoptotic activity just as a wild-type would but it is not suppressed by BARD1 expression (18). Thus, the domain is necessary for BRCA1-BARD1 heterodimerization but not for BRCA1-mediated apoptosis.

To test this concept in BARD1, researchers deleted the first ninety-five residues on the BARD1 protein, which contains most of the dimerization site (18). They found that in these mutants, BARD1 is unable to bind BRCA1 and the cell undergoes BRCA1-mediated apoptosis. Thus, the dimerization domain is also necessary on BARD1 for BRCA1-BARD1 heterodimerization.

Studies in past years have shown that where BARD1 is expressed in the cell determines BARD1’s apoptotic role (16,17,18,19,21). Specifically, if it is localized in the cytoplasm, then the cell will undergo apoptosis (19,21). Recently researchers studied the same possibility for BRCA1 (8,17,18,19). Normally BRCA1 is sequestered in the
nucleus, so the researchers proposed a link between nuclear export of BRCA1 and cytoplasmic apoptosis mediated by BRCA1.

To study this hypothesis, they manipulated only the BRCA1 protein in various ways—BARD1 was not used in this study. First, they deleted the first 170 amino acids of the BRCA1 protein (18). Within this sequence is the nuclear export signal that allows the protein to pass through the nuclear membrane into the cytoplasm. They found that removing this signal reduced the levels of apoptosis, implying that the truncated BRCA1 was unable to leave the nucleus.

Secondly, they deleted the first seventy amino acids of the BRCA1 protein, which deleted the BRCA1-BARD1 dimerization domain, but the truncated BRCA1 was still able to export from the nucleus (18). Thus the first seventy amino acids are not necessary for nuclear export and do not contain the nuclear export signal. The truncated BRCA1 is also still able to induce apoptosis in the cytoplasm, so the first seventy amino acids do not contain any necessary sites for the apoptotic role. However, the first seventy amino acids are necessary for BRCA1-BARD1 dimerization. This evidence suggests that the first seventy amino acids of BRCA1 may serve only as a dimerization domain for BARD1.

Lastly, they removed the nuclear export signal from its original site and artificially relocated it to the C-terminus of the protein (18). When they did this, the cell had nearly normal levels of apoptosis, even in the presence of BARD1. This was sufficient evidence that the nuclear exporting signal is necessary for BRCA1 to leave the nucleus and induce apoptosis in the cytoplasm. This fits with the current model of
BRCA1-mediated apoptosis, which occurs by activating an apoptotic cascade beginning with polyubiquitination and including proteosome-mediated cellular disassembly (18). Since the proteins being ubiquitinated can not enter the nucleus, BRCA1 must be able to travel from the nucleus to the plasma membrane in order to induce apoptosis by this pathway.

Recently researchers have confirmed that BARD1 is responsible for nuclear retention of BRCA1 (17,18,21). They devised a study in which CRM1 (chromosome region maintenance 1) was co-expressed with BRCA1 and BARD1 (18). CRM1 is the export receptor on the nuclear membrane that facilitates BRCA1 exportation to the cytoplasm (18). Co-expression of CRM1 with BARD1 resulted in no increase in apoptosis, implying that BRCA1 was not leaving the nucleus (18). The explanation for this is that when BARD1 binds BRCA1 through the dimerization domains, the length of the BARD1 protein is sufficient to block access of the nuclear exporting signals, which are within 100 amino acids of the BARD1-BRCA1 dimerization domain. Even though BARD1 does not directly bind the export signal domain, it prevents any other proteins from binding and thus retains BRCA1 in the nucleus.

Lending additional support to this hypothesis, when the nuclear export signal is relocated to the C-terminus and CRM1 is expressed, BRCA1 is exported from the nucleus and induces apoptosis by its cytoplasmic mechanism (18). BARD1 is unable to block the nuclear export receptor when it is relocated to the C-terminus, indicating that BARD1’s mechanism of interference is structural and position-dependent.
Researchers recently studied the precise BRCA1 region necessary for BARD1 binding (17,18). Four BRCA1 sequences of limited amino acids (1-304, 1-110, 1-70, and 70-180) were paired with BARD1 (18). Only the 1-304 and 1-110 BRCA1 segments competitively bind BARD1, indicating that the entire 1-110 sequence is necessary for binding, not just the RING motif. Thus, even though direct binding between BARD1 and BRCA1 is at the RING motif, interactions along the entire 1-110 BRCA1 sequence are necessary for stability and complete function.

A potential side benefit of these truncated BRCA1 sequences is that they may bind BARD1 and permit full-length, completely functional BRCA1 to shift out of the nucleus and induce apoptosis. The truncated sequences would thus act as inert decoys to bind BARD1 and ‘keep it busy’ while full-length BRCA1 is permitted more time to float around in the nucleus and arrive at a nuclear export membrane protein, where it is shuttled to the cytoplasm. Therefore a possible breast cancer treatment may include administering exogenous BRCA1 N-terminus sequences to bind BARD1, allowing endogenous BRCA1 to export to the cytoplasm and activate apoptosis in cancer cells.

Interference of BARD1 expression by siRNA caused a modest increase in apoptosis in recent studies (17). This too could be used in a similar fashion as the exogenous BRCA1 N-terminus sequences to prevent BARD1 from binding BRCA1 and thus increasing cytoplasmic BRCA1 levels.

One of the most intriguing results in research over the past year included work that indicated a compensatory mechanism in BRCA1/BARD1 complexes (18). Specifically, researchers observed that an exogenous decrease in either BARD1 or
BRCA1 causes a compensatory down-regulation in the other protein (18). It was not determined if the proteins up-regulate when the concentration of the other protein increases. Thus blocking either BRCA1 or BARD1 may only temporarily create an imbalance that favors apoptosis, as equilibrium will eventually be restored.

If BRCA1-BARD1 equilibrium is restored in this situation, the prospective treatment may ultimately be harmful to a breast cancer patient because the resulting cellular environment would be anti-apoptotic, and there would be less BRCA1 in the cell to promote HR. Thus, a more conceptually appealing treatment may include exogenous BARD1 or BRCA1 supplementation, increasing levels of one to promote apoptosis. This may only be temporary if up-regulation occurs, however, requiring constantly increasing levels of a BARD1 or BRCA1-boosting drug. The consequences of artificially high levels of BARD1 or BRCA1 in cells are not known, indicating the need for extensive clinical testing of any potential drugs.

If exogenous supplementation aids in reducing or reversing breast cancer growth, decisions about BARD1 versus BRCA1 supplementation may hinge on the status of p53 in the cells—if p53 is defective, the p53-dependent mechanism of BARD1-mediated apoptosis will be ineffective and thus BARD1 supplementation in this instance will be ineffective. In that case, BRCA1 supplementation would be a superior treatment.

The BRCA1-BARD1 heterodimer equilibrium reflects the more general theme of human physiology: homeostasis. Manipulating this equilibrium may prove effective in treating breast cancer with molecular targeted therapies. While it seems unlikely that a cure for breast cancer will arise from this potential therapy, it may become a useful
adjuvant therapy to established therapies such as chemotherapy, radiation, and surgery, or perhaps as a more tolerable alternative therapy. Additionally, the BARD1-BRCA1 equilibrium may be evaluated as a diagnostic tool in measuring the extent of apoptotic activity in breast cancer cells. In other words, the BARD1-BRCA1 ratio and concentrations in the cell could serve as a barometer for apoptotic activity in cells. This could result in a more specific grading of a tumor’s aggressiveness and more appropriate traditional treatment.
BRCA1 & CHK2 INTERACTIONS

Checkpoint kinase 2 (CHK2) aids BRCA1 DNA repair by two functions: most directly, CHK2 phosphorylates BRCA1 and activates it for HR-mediated DNA repair (4,12,22,23). Secondly, once activated by ATM, CHK2 phosphorylates Cdc25 (a cell cycle activator), providing a binding site on Cdc25 for 14-3-3 adapter proteins (4,12,22,23). These adapter proteins block Cdc25 function and thereby arrest the cell cycle at the G2 checkpoint (pause during the second growth period of the cell cycle). Mitosis does not occur. This aids BRCA1 function by generating a pause in the cell cycle so BRCA1 can repair the DNA in a static, stable cellular environment and so the DNA mutation is not reproduced in daughter cells.

In response to DNA damage, the BRCA1 protein becomes rapidly hyperphosphorylated at multiple sites by several kinases including ATM and CHK2 (4,12,23). ATM is the gene mutated in the ataxia telangiectasia syndrome (ATS). ATS is characterized by cerebellar degeneration, immunodeficiency, chromosomal instability, cancer predisposition, radiation sensitivity, and cell cycle abnormalities. The disease is genetically heterogeneous, with four complementation groups that have been suspected to represent different genes (22). The ATM protein is critical for the cellular response to DNA damage by regulating the G1 (first growth phase), S (replication phase), and G2/M (transition from second growth phase to mitosis) cell cycle checkpoints and by the phosphorylation of an array of protein substrates, including CHK2 (22,23). Therefore, the CHK2 protein kinase governs checkpoint responses by an ATM-dependent mechanism (12,22,23). Mutation of the BRCA1 target sites for ATM, serines 1423 and
1524, abolishes the ability of BRCA1 to mediate the G2/M checkpoint, while mutation at serine 1387 disrupts the S-phase checkpoint (22,23).

Mutations of the CHK2 gene have been found in a subset of patients with Li-Fraumeni syndrome (a rare autosomal dominant predisposition to breast cancer, sarcomas, and other neoplasms in children and young adults, caused by an alteration in the p53 tumor suppressor gene) and with familial breast cancer (23). BRCA1 is phosphorylated by CHK2 on serine 988, and mutation of this residue prevents the dispersion of BRCA1 from subnuclear foci after recombination (22). Therefore it disrupted both the BRCA1-dependent promotion of HR and the suppression of NHEJ. Epidemiological evidence has implicated CHK2 and BRCA1 in the same breast cancer prevention pathway, but the molecular process controlled by their interaction has not been identified (23).

The serine 988-to-alanine substitution (S988A) eliminates phosphorylation by CHK2 (this manifests itself by failure to promote HR and failure to form a recombination complex with Rad51). Inhibition of CHK2 kinase activity mirrors the effects of the BRCA1 S988A mutation, reaffirming that CHK2 binds and activates BRCA1 through serine 988 (22,23).

In one epidemiological study, the 1100delC mutation of CHK2 (deletion of cysteine 1100) was linked to an increased incidence of familial breast cancer in noncarriers of BRCA1 germ line mutations, while no increased CHK2 mutation frequency was found among individuals with BRCA1 mutations (24). This provides evidence that BRCA1 is downstream of CHK2 in the tumor suppression pathway,
confirming previous theory. This also suggests that if the DSBR pathway controlled by BRCA1 and CHK2 is already inactivated by BRCA1 mutations, then abolishing CHK2 function may confer no demonstrable additional risk of disease. Furthermore, in this case, restoring CHK2 from a mutated or damaged state would be futile as long as BRCA1 remains mutated or damaged.

The novel finding in recent studies is that both Rad51-dependent DSBR and the accumulation of the Rad51 recombination complex requires the BRCA1 serine 988 residue, which is a target for CHK2 phosphorylation, therefore suggesting that CHK2 protein kinase activity on BRCA1 is instrumental in dictating its functions in HR (12,22,23).

In summary, CHK2 activation of BRCA1 promotes Rad51-dependent HR while simultaneously inhibiting NHEJ. The promotion of HR and suppression of NHEJ are governed by the serine 988 residue of BRCA1, which is phosphorylated by CHK2.

Thus a significant conclusion from 2004 research of BRCA1-CHK2 interactions is that the CHK2 phosphorylates BRCA1 at serine 988 to simultaneously promote Rad51-dependent HR and inhibit error-prone NHEJ (12,22,23). This differs from previous theory that assigned BRCA1-CHK2 to the role of simply choosing either HR or NHEJ, neglecting the dual role of promoting one and inhibiting the other.

BRCA1-CHK2 interactions may lead to breast cancer diagnosis, treatment, and prevention technologies. Assessing CHK2 genotype in addition to BRCA1 through genetic testing may prove worthwhile in assessing the breast cancer risk of patients or the aggressiveness of an existing cancer. CHK2 activators, including exogenous ATM
supplementation, endogenous ATM stimulation, or synthetic ATM-like activators may play a contributory role in treating breast cancer if BRCA1 and the other downstream components remain intact and functional. Deficiencies in CHK2 activity identified through diagnostic methods may be corrected by preventive CHK2-activating supplementation. Lastly, exogenous CHK2 supplementation may simultaneously address the cellular needs of halting the cell cycle and initiating HR through BRCA1. This prospective therapy would likely be most beneficial for patients with compromised CHK2 levels and an otherwise functional DSBR pathway.
CONCLUSION

The initial years of 21st century oncology have revealed a noticeable trend toward molecular cancer therapies, and breast cancer is no exception. While current focus in some ways remains on estrogens and estrogen receptors (Tamoxifen, Arimidex, etc.), research performed currently on BRCA1 and other components of the apoptosis and DNA repair pathways will hopefully come to fruition in the next several years as a new generation of specific, targeted breast cancer therapies.

Although the therapies require extensive research and development, diagnostic methods may be in the more immediate future, providing clinicians with the ability to more precisely diagnose the grade and stage of a tumor, and ultimately even the biological flaw fueling the uncontrolled abnormal growth. The 2004 literature reviewed in this thesis suggests that identifying and measuring the status and activity levels of BRCA1, BACH1, BARD1, and CHK2 for diagnostic purposes is currently more plausible than manipulating those proteins for therapeutic purposes. Accurate assessment of risk profiles and appropriate prevention measures may also generate a substantial reduction in breast cancer mortality and confine the disease to a nuisance rather than a tragedy. In other words, technology that informs patients and clinicians of when cancer will arise and how aggressively it will grow may be as powerful as technology that actually treats the cancer.

For cancer researchers, scientific work is becoming increasingly business-oriented. A recent interview with Dr. Nira Ben-Jonathan, a breast cancer researcher at
the University of Cincinnati Vontz Center of Molecular Studies, reveals that a lab’s direction with various projects often becomes entangled with agendas and expectations.

One study incorporating the role of prolactin in a breast cancer cell line exemplifies this challenge: preliminary research revealed promising results, and the lab researchers debated the appropriate next step—continue building evidence for a clinical correlation and publish the results as soon as possible, or delve further into the biological underpinnings of the mechanism and publish the results later when more is known. Publishing sooner would guarantee that they would have a novel piece of research in the public eye and aid their pursuit for further research funding. Publishing later may include more thorough, higher quality, and more beneficial research to society, but if another lab beats them to the results then they will have nothing to show for their work.

As a result of the current incentive structure in basic science research, the lab decided to publish sooner. They rationalized the decision by agreeing that even with the initial research in the public eye and welcoming competing labs to find the biological mechanism, their lab will have a head start in the pursuit by having the equipment and animal subjects ready for immediate research.

This research climate motivates researchers to produce meaningful results in timely fashion but also forces them to act in a more short-sighted manner than they would ordinarily prefer. Short-term mechanistic studies are more popular than 5-, 10-, or 20-year epidemiological studies, for instance. Whether this is good or bad for the field may be debated—either way it is an issue that researchers must contend with daily.
Public policy will play a vital role in both encouraging productive, beneficial basic science research and prevention efforts for the general population. Knowledge is one of the most powerful tools for fighting breast cancer in society, and saving lives will require both the advancement of laboratory research and dissemination of basic information to the general population. While the United States government currently funds a majority of cancer research through agencies such as the American Cancer Society and National Institute of Health, private organizations such as the Susan G. Komen Breast Cancer Foundation have assumed the bulk of responsibility for informing the public about routine breast screening and other prevention measures. In the future, public agencies may be wise to consider a balance in public funding between fundamental research and public awareness efforts.
Figure 1. Diagrammatic representation of BRCA1 protein highlighting the N-terminus location of the RING domain (RING) for BARD1 binding, the C-terminus BRCT domain (BRCT) for BACH1 binding, the DNA binding domain (DNA) necessary for BRCA1 to bind and repair damaged DNA, and the nuclear export sequence (NES) that must be accessible for BRCA1 to be exported from the nucleus into the cytoplasm of the cell. The BARD1 protein (BARD1) and BACH1 protein (BACH1) are also depicted, making contact with the BRCA1 protein in the approximate location of their respective binding sites.
Figure 2. Diagrammatic representation of BRCA1-BARD1 interactions in the cell.

When BRCA1 and BARD1 heterodimerize in the nucleus, both proteins are retained in the nucleus. DNA repair results once BRCA1 binds the other components of the DNA repair complex (BRCA2, RAD51, BACH1) and dissociates from BARD1. When either BRCA1 or BARD1 are unbound and independent, they have the ability to leave the nucleus. When in the cytoplasm, both BRCA1 and BARD1 can independently initiate apoptosis. BARD1 requires p53 to initiate apoptosis in the cytoplasm. Conversely, BRCA1 initiates apoptosis by a p53-independent mechanism. Thus both BARD1 and BRCA1 are capable of initiating apoptosis when they are located in the cytoplasm.
REFERENCES


