You're Under Arrest, But Breast Cancer Is Not!

Student group names kept anonymous
Departments of Art and Biology, Lake Forest College, Lake Forest, IL 60045, USA

Breast cancer is a cellular disease, the result of aberrant cell growth starting in breast tissue. Breast cancer affects both men and women, the disease has many symptoms most of which occur as abnormalities in the breast tissue. Inherited mutations in the BRCA1 and BRCA2 genes, silencing of the 14-3-3-sigma gene, or the amplification and overexpression of the HER-2 gene can ultimately lead to breast cancer. Although it was known that families with a history of breast cancer were missing both BRCA proteins, that HER-2 is a proto-oncogene, and that 14-3-3-sigma play a significant role in G2 arrest the molecular mechanisms were unknown. This review will illuminate the biological functions of the BRCA proteins and why they cause cancer. The relationship between the 14-3-3-sigma gene, p53, and cell cycle arrest and its implications in breast cancer will also be analyzed as well as why many normal cells express HER-2 at low levels but in some forms of breast cancers the HER-2 is amplified and over expressed. A greater understanding of the genes involved in breast cancer will hopefully lead to more advanced treatments such as gene therapy.

History

Breast cancer is a cellular disease, the result of aberrant cell growth starting in the breast tissue (1). There have been many insights into the causes of breast cancer and many epidemiological studies that provide a relationship between environmental, racial, and cultural influences in the occurrence of cancerous cells (2).

In the eighteenth century, there was a significant outbreak of breast cancer (3). This outbreak was recognized by physician LeDran who observed that breast cancer spread through the lymph nodes, one of the major symptoms of breast cancer today (3). The causes of breast cancer are still unknown, however the ideas of mutations in healthy cells are very apparent.

Symptoms

As breast cancer progresses, many physical changes occur within the body. The most probable and apparent symptom associated with breast cancer is a new lump or mass in the breast. If the lumps are painless, irregular in shape, or hard it is possible that it is cancerous. As cancer progresses, other symptoms occurs include swelling in the lump area, skin irritation, pain or redness of nipple, nipple discharge other than breast milk, or a lump found in the underarm area (1). Detection and diagnosing an individual is the most important step with cancer.

Breast cancer has four distinct stages. Stage I is considered the early stage, in which the tumor is no more than one inch. Stage II can be considered the time in which the tumor has spread to the lymph nodes. Stage III, locally advanced cancer, means the tumor is larger than those found in Stages I and II. Stage IV is metatistic cancer, in which, the cancer has spread to other parts of the body. At the conclusion of Stage IV, individuals have the choice to undergo treatment or to remove the cancerous tumor. (1)

Epidemiology

Breast cancer affects many individuals around the world. In the year 2002, it is estimated that 203,500 new cases of invasive breast cancer will be diagnosed among women in the United States alone (1). Breast cancer affects men as well, it is estimated that 1,500 new cases of breast cancer will arise among men in the year 2002 (1).

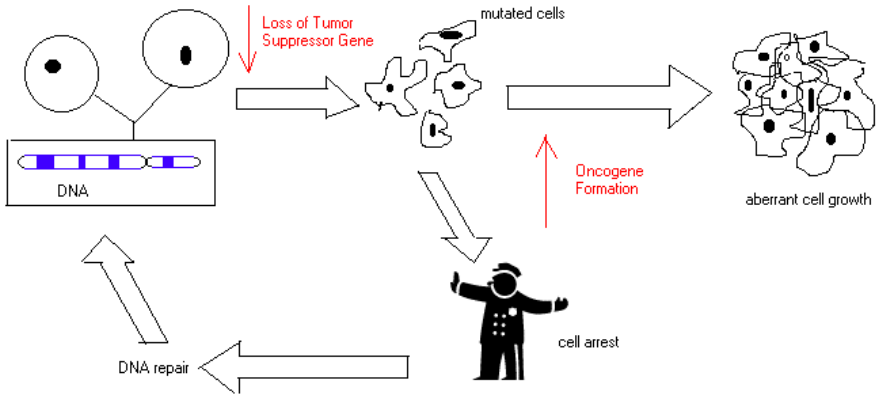

Figure 1**: Road Map to Breast Cancer:** Normal cells found in breast tissue undergo mutations caused by environmental or heredity factors. With cell cycle in control the mutated cells stop dividing and undergoes cell arrest, in which DNA repair can take place. However with breast cancer, there is a loss of tumor suppressor genes, which help to inhibit aberrant cell growth, along with oncogene formation.

Breast cancer is rarely found in women under the age of 25, unless there has been a family history of it (4). There is variation in life expectancy, as well as possible reoccurrence, of women diagnosed with breast cancer.

There are two genes implicated with breast cancer, which account for the inherited form of the disease. These genes are called BRCA1 and BRCA2, and account for 83% of breast cancers occurring by age 70. However, only about 20% of women with familial history of breast cancer carry these particular genes (4).

Pathology

The development of breast cancer is accompanied with pathological signs with the breast. Females with larger breasts have more have more fat surrounding possible tumor growth, making it harder to detect (4). Beginning in the breast tissue, malignant tumors are able to metastasize to other organs of the body, making breast cancer so deadly.

Biological Basis

The biological basis of breast cancer is significantly related to breast cancer susceptibility genes, BRCA1 and BRCA2. The majority of breast cancer cases do not develop cancer because of heredity, but because of a mutation in the DNA of a normal cell. Only 5-10% of breast cancer cases are due to heritance of BRCA genes. (5).

The link with BRCA genes can be understood by the mutated genes are passed to the offspring, thus making the offspring more susceptible to breast cancer.

Gaps in Knowledge

Scientists and doctors knew that BRCA proteins were the only genes implicated with breast cancer thus far. Families that had hereditary breast and ovarian cancer were missing both the BRCA proteins. Scientists wanted to find the missing between with BRCA proteins and breast cancer.

There also have been associations made between the Her2 and breast cancer. Her2 was first discovered as an oncogene in 1984 when it was isolated from independent neuroglioblastomas that had developed in carcinogen-treated rats (6). Even though Her2 was identified as an oncogene, and was thus connected to cancer, there was not a direct connection made with its role in breast cancer at the time.

The 14-3-3-sigma gene also did not have any real implications with cancer directly, except for its help with the G2 arrest, which would be involved with the slow formation of cancer. There was no real link between the 14-3-3-sigma gene and breast cancer at all as of 1999, when this particular gene was first involved with cell cycle arrest.

BRCA1 and BRCA2

It has been found that inherited mutations in BRCA1 and BRCA2 influence breast, ovarian, and other cancers (7)**.** Therefore the main function of this review is to examine what is known about the biological functions of the BRCA proteins and find out why they cause cancer (7).

BRCA1and BRCA2 instructs large proteins that are found in a variety of places in different tissues during the S and G2 phases (7).

The main purpose of these two proteins is to maintain chromosome structure through the use of DNA strand break repair (DSB repair) (7). At this point in time, this is the only relevant thing known about them because they take part in so many processes in the cell (such as DNA repair and recombination, cell cycle control, and transcription) but we are not exactly sure what they do in the processes (7). The role of the BRCA proteins in the protection of chromosome structure stems from diverse functions in biological response to DNA damage (7). When cells enter G2 arrest they are held there and repaired by either BRCA1 or BRCA2. Cancer patients lack this gene therefore there DNA is not being repaired at all or it is simply being repaired incorrectly, because that is the easiest thing for the cell to do.

BRCA1 and BRCA2 were identified about six years ago through analysis of families at high risk for breast or ovarian cancer. About 10% of breast cancers case clusters in families; some are due to highly penetrant germline mutations in one or another of a small number of genes, such as BRCA1 and BRCA2, giving rise to high cancer risk. Germline mutations in BRCA1 and BRCA2 account for about 15% to 20% of observed risk, but are found in over 80% of the families where six or ore cases occur (7).

BRCA2-RAD51 Complex

BRCA2 is 3,418 amino acids (aa) long, which is almost twice the size of BRCA2 (1,863 aa). It contains eight repeats of the of the ~40 residue BRC, and it also contains nuclear localization signals (NLS), which mean that it is found in the nucleus. It is known that BRCA2 binds directly with RAD51 (7), a mammalian homolog RecA that is essential for DSB repair through recombination (8). BRCA2 binds to RAD51 through the conserved BRC repeat region, in which RAD51 can bind with six out of the eight residues (8). It is known that homologous recombination (HR) and non-homologous end joining (NHEJ) contribute to DNA strand break repair (DSBR) (9).

Soon as damaged DNA reaches a checkpoint BRCA2 begins to work immediately. DNA strand breaks (DSBs) activate signal mechanisms, which attach to the DSBs and cut the damaged tracts back a little bit more through the use of exonuclease activity. This creates a single strand DNA (ssDNA) tract. Than through the phosphorylation of DNA damage-signaling kinases (ATM or ATR) they activate the BRCA2-RAD51 complex to the location of the damage (7). The BRCA2-RAD51 complex causes the bottom strand of the damaged DNA to bind with the top strand of the other alpha helix. Then this causes the top strand of that alpha helix to bind with the bottom strand of the damaged DNA. Then the BRCA2-RAD51 complex is removed through dephosphorylation. This allows for the good strands of the DNA, from the second alpha helix, to be used as a template for the damaged alpha helix. After they are through coding, the repair is fixed and the DNA is fine.

Accepted Roles of BRCA1

BRCA1 is 1,863 aa long, which is half the size of the BRCA2 protein (3,418). It also contains a RING domain that binds with the BARD1 protein, a de-ubiquitinating enzyme (10). There is a NLS that binds with the RAD50 protein, which is responsible for bringing about end joining.

In inherited breast and ovarian cancer, the BRCA1 gene appears to be altered frequently (11), therefore less is known about how BRCA1 may work. It is known that BRCA1 does not perform one large task, but instead several small ones that add up to DSB repair. There are several different theories of how the BRCA1 protein might react in response to damaged DNA (7).

The sensing and checkpoint control of the cell cycle assumption states that BRCA1 can perform the activity of regulating the length and persistence of ssDNA generation at sites of DNA breakage. BRCA1 works in the same way that BRCA2 does, except it used a variety of different signaling mechanisms. BRCA1 does not bind with RAD51, instead it will bind with MRE11, RAD50, or Nbs1 proteins in order to complete this function.

The DSB resection assumption states that a role of BRCA1 may be important when directing DSB repair down these routes. BRCA1 actually helps the resectors in the process of cleaning up the damaged DNA before the signal mechanisms are phosphorylated.

The altering DNA topology at damaged sites assumption states that BRCA1 might perform activities at DSB sites through its interaction with enzymes that alter chromatin and DNA structure. It will basically work wit enzymes such as SWI/SNF, BACH1 and HDAC order to change the structural shape.

The transcriptional regulation assumption states that BRCA1 plays the role of connecting DNA damage-sensing and response

mechanisms are well illustrated by its function in regulating the expression GADD45, a tumor suppressor gene that works like the p53 gene. What this process basically does is the BRCA1 uses theGADD45 to stop the damage DNA from entering the next phase in the cell cycle, which in turn gives plenty of time for the damage to be repaired.

The transcription-coupled repair assumption and the BRCA1/BARD1, as a ubiquitin ligase, assumption actually goes hand in hand. BARD1, a protein that interacts with BRCA1 through the N-terminal RING domain, is thought to be able to control RNA processing after the DNA damage has occurred. The reaction between the two causes a much needed to restrain the DNA damage. Therefore it is believed that ubiquitin ligase activity of the BARD1-BRCA1 may be involved to target deprivation of proteins that carry out RNA processing (7).

The major problem with the BRCA proteins is that very little is known about the role of them as well as the role they play in preventing breast cancer. Therefore without greater research is unlikely that recent progress in defining the functions of these proteins can be translated into advances in the prevention or treatment of cancer (7).

p53 and Cell Cycle

The failure of DNA damage to be corrected in cellular reproduction is one of the main reasons for cancer. There are many genes that can correct DNA damage, and subsequently prevent aberrant cell growth. DNA damage causes an increase in the concentration of the gene p53, a tumor suppressor gene (12).

As of 1997, there has been a significant finding of a new gene, 14-3-3-sigma, that is connected with p53. This gene is involved with the human cell cycle, arrest at the G2 check point.

14-3-3-sigma

Upon the study in 1997, scientists used a DNA damaging agent, such as γ-radiation, to damage the DNA of human breast cells (12). The damaged DNA triggers the p53 gene, and in turn activates the 14-3-3-sigma. This gene sequesters cyclin dependent kinases (CDK), which are involved DNA replication. If these Cdk's are unable to enter the nucleus of a cell, it is unable to undergo DNA replication. This process is necessary to inhibit aberrant cell growth, which is one of the main causes of breast cancer. (14).

Overexpression of the 14-3-3-sigma obstructs entry into the cell cycle by inhibiting Cdk activity in breast cancer cells, as well as inhibiting cell proliferation (15).

14-3-3-sigma is involved with mitotic catastrophe, or apoptosis. In order to determine the role of 14-3-3-sigma in apoptosis, scientists added adrimcyin, a DNA-damaging agent, that either expressed or did not express 14-3-3-sigma. Cells that expressed14-3-3-sigma became enlarged and exhibited properties characteristic of cells in the G2 cell arrest phase. Cells that did not contain 14-3-3-sigma had highly condensed chromatin, suggesting a short life span, or apoptosis (16). If these cells are undergoing cell death, then they will not be able to enter the nucleus and transcription can not take place. The idea of the inhibition of aberrant cell growth thus comes into play.

Gene Silencing

Gene silencing is an interesting factor that has recently been implicated with breast cancer, and 14-3-3-sigma. Silencers cause chromatin to coil up into a condensed, inaccessible form, ultimately inhibiting transcription (17). In the case of breast cancer, gene silencers can be promoted through the process of methylation. In the year of 2000, scientists discovered information that led to the notion that hypermethylation of 14-3-3-sigma will lead to breast cancer.

Scientists found that hypermethylation of the CpG islands, found in the promoter area of 14-3-3-sigma, were detected in 91% of human breast cancer cells (18). The loss of 14-3-3-sigma thus leads to tumor formation and breast cancer.

In this study, Ferguson, *et al*. did an analysis of 14-3-3-sigma expression. They found that eleven normal breast cell lines all had sigma gene expression. However, that was not the case for breast cancer cell lines. In nineteen primary breast carcinomic cell lines there was no sigma mRNA found, thus providing evidence for the relationship between 14-3-3-sigma methylation and breast cancer (18).

The increase of methylation of the CpG islands suggests that malignant progression of breast cancer involves an accumulation of mutations that contribute to the silencing of tumor suppressor genes, such as the 14-3-3-sigma (19). 14-3-3-sigma was initially implicated in cell cycle arrest. With new information about gene silencing and methylation, it can be understood why 14-3-3-

sigma is associated with the loss of expression in human breast cancer formation.

There have been many treatments with 14-3-3-sigma and breast cancer. 5-aza-2'deoxycytidine is an anti-tumor agent, which aids the reactivation of tumor suppressor genes. Also, deoxycytidine, a kind of DNA methylase helps to block DNA methylation. (21). 5-aza-2'-deoxycytidine is needed for the reexpression of a silent gene in order to combat breast cancer.

In 2000, scientists completed a study on the effects of this drug with 14-3-3-sigma. Two cancerous cell lines containing fully methylated genes that did not express 14-3-3-sigma had the methyltransferase 5-aza-2'-deoxycytidine added. After a few days, 14-3-3-sigma mRNA was re-expressed. These results help support that methylation is responsible for the loss of 14-3-3-sigma transcription in breast cancer cells. (19).

HER-2

The human epidermal growth factor receptor 2 gene encodes HER-2 HER-2 is an epithelial cell surface receptor that expresses tyrosine kinase activity (22). In normal cells, it acts as a receptor for cellular growth factors, thus regulating specific areas of cell growth and division (22). In some cancerous cells the protein loses its normal response to other regulatory proteins, which leads to unregulated cell growth (22).

In many normal cells, HER-2 is expressed at low levels, but in 20-30% of human breast cancers, the HER-2 proto-oncogene is amplified at the genomic level and overexpressed at the protein level (22,23). The presence of Her-2 in tumors is a predictor of worse outcome and shortened survival in breast cancer patients (24).

As of 1999, current research demonstrated the HER-2 gene encodes a receptor-like tyrosine kinase (p185HER-2) that plays a very integral role in the development of several human carcinomas. P185HER-2 has a common structural organization, similar to other epidermal growth factor receptor (EGFR) family members (25).

Dimerization of receptor tyrosine kinases is normally induced by ligand binding, and is essential for their activation and for successive steps in signal transduction (25). P185HER-2 shows constant activity, even in the absence of a ligand(25). This is enhanced by the overexpression of normal p185HER-2 (25). The most common mechanism that transforms HER-2 into an oncogene is the overexpression of normal p185HER-2.

Herstatin is a secreted protein that exhibits a high affinity to p185HER-2. Herstatin mRNA is expressed in normal human fetal kidney and liver cells and at reduced levels relative to p185HER-2 mRNA in carcinoma cells that contain an amplified HER-2 gene (25). Herstatin is encoded by the alternative splicing of HER-2.

It has been concluded by Doherty *et al*. that herstatin is a naturally occurring inhibitor of p185HER-2; it disrupts dimer formation, reduces the tyrosine phosphorylation of p185, and inhibits the anchorage independent growth of cells that overexpress HER-2 (25). Although more studies need to be done it is quite possible that herstatin could have various therapeutic effects against human cancers that occur as a result of the overexpression of p185HER-2 (25).

p53 and HER-2

Overexpression of the HER-2 oncogene is also associated with either proliferation or differentiation and apoptosis (26). Recently, the role of p53 on these different outcomes was investigated.

A pathway known as the MAPK pathway is implicated in many cell systems in the growth and transformation of cells. It is also one of the pathways that may activate downstream HER-2 (26). There are several substrates of the MAPK pathway, including transcriptional factors Jun, Fos, and c-Myc, which are thought to activate not only cell cycle machinery but also cell cycle checkpoints (26).

Previous in vitro studies have shown that the transfection of HER-2 into cellular models has induced proliferation and a malignant phenotype (26). Recently, however Casalini *et al*. reported that HER-2 transfection resulted in decreased growth rate, inhibition of entry into S phase, differentiation, and/or a growth inhibitory effect in some cell lines (26).

More specifically, in breast cancer cell lines, this mechanism of growth inhibition and differentiation through HER-2 receptor activation has been suggested to involve p53 (26). HER-2 overexpression is often found in breast tumors with p53 mutations (26).

The significance of p53 status for the activity of HER-2 in promoting proliferation using HER-2 transfected cells differing in p53 status was tested (26). The results showed that p53 status does indeed greatly influence the effect of HER-2 overexpression in tumor cells in

promoting either proliferation or differentiation and apoptosis. It also shows how important p53 regulation is in the HER-2 signaling pathway. The data suggest that the repair of p53 function might counteract the malignancy of tumors associated with HER-2 over expression.

Bombesin antagonists effects on HER-2

Another thing that has been proven to reduce tumor volumes is antagonists of bombesin. Bombesin is a neoropeptide that is released by the neurons. Previously, it has been shown that antagonists of bombesin (BN)/ gastrin-releasing peptides (GRP) are able to inhibit the growth of various types of cancers by interfering with the growth-stimulatory effects of BN-like peptides and down-regulating epidermal growth factor receptors on tumors (27). Recently, the involvement of bombesin/ gastrin-releasing peptides and epidermal growth factors (EGF) in the development and progression of breast cancer has been investigated (27). It has been suggested that new therapies aimed at interference with these growth factors or their receptors could be developed (27). Neuropeptides (including BN and GRP) act as morphogens for some normal and neoplastic tissues (including breast tissue) and bind to cell surface receptors (27).

BN/GRP antagonists are able to affect HER-2 in mammary tumors. Both can significantly inhibit the growth of MDA-MB-435 (human estrogen-independent breast carcinoma) cancers. GRP receptors were detected in MDA-MB-435 cancers, thus showing that they do mediate the inhibitory effect of the antagonists (27).

The expression of BN/GRP receptors on tumors was analyzed (27). It was found that BN/GRP antagonists RC-3095 and RC-3940II were able to significantly reduce tumor volumes (27). This tumor inhibition was associated with a considerable reduction in the expression of mRNA and protein levels of the ErbB/HER-2 receptor family (27). A decrease in the expression of c-jun and c-fos oncogenes was recorded as well (27).

BN antagonists can produce a reduction in the expression of protein levels of every member of the ErbB/HER receptor family as well as a decrease in the expression of the c-jun and c-fos oncogenes (27). Also, BN-like peptides can up-regulate EGFRs in various cancers, but BN antagonists can block this action (27). Consequently, BN/GRP antagonists seemingly inhibit MDA-MB-435 cancers by blocking the effects of the BN-like peptides and also inducing a down-regulation of the HER system (27). Ultimately, BN/GRP amtagonists are able to reduce tumor volume and inhibit the growth of various cancers by interfering with growth stimulatory effects (27).

Treatments and Cures

There have been multiple treatments with breast cancer. The most popular treatments include lumpectomies and mastectomies. Auxiliary dissection is another treatment that entails detecting the cancer that has spread to the lymph nodes (1). Possible cures for breast cancer include radiation, in which the tumors are killed with UV radiation and chemotherapy, the most famous treatment in which chemicals in order to kill cancerous cells.

As of 2002, several new treatments have started to be more diligently employed than treatments in the past. Tumor ablation is where cancer can be frozen or vaporized with lasers or high-energy radio waves. These are delivered through probes that are inserted into a little slit into the breast, in which the probe opens up like an umbrella. Endoscopy is when tumors can be observed with a tiny camera, inserted into a milk duct in the center of the nipple. Targeted radiation is when a tiny radioactive bead is delivered into the tumor site (after a lumpectomy) through a small balloon-tipped catheter. Smart drugs are a new generation of drugs that bind to specific receptors or block particular proteins (28).

In the Future

Reinsertion of the BRAC1 and BRCA2 proteins to cancer patients who are lacking the proteins, will hopefully stop the inherited loss of the proteins in the future. Other drugs can be discovered to inhibit methylation of 14-3-3-sigma. Treatments that use herstatin, repair p53 function, and/or use BN/GRP antagonists can be implicated in patients with breast cancer that overexpress HER-2.

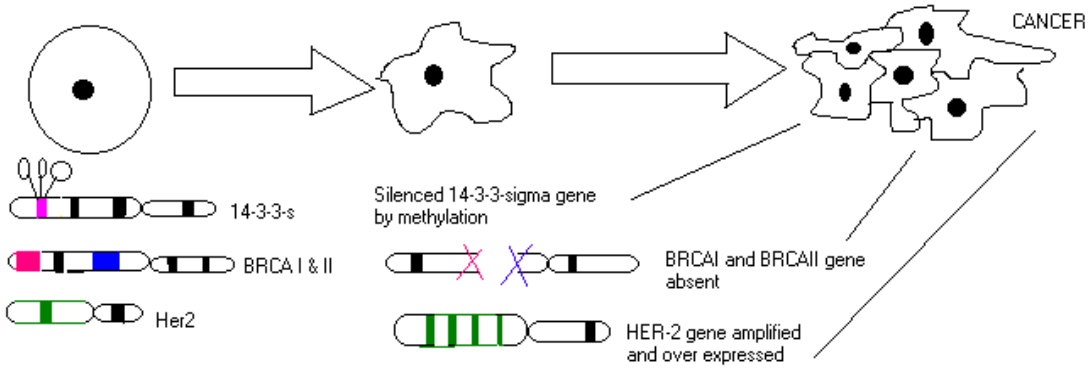

Figure 2**: Newly Revised Model Breast Cancer:** There are three new genes, which are involved with breast cancer. Cells that contain mutations in these genes can ultimately lead to breast cancer. The 14-3-3-sigma gene is silenced by methylation, BRCA I and II genes are absent, and the overexpression and amplification of Her2 all lead to breast cancer.

Wrap It Up

Breast cancer is a disease that affects many individuals. Although significant process has been made in determining the factors that may lead to cancer. (See Figure 2). Current Research is focusing on understanding the mechanisms involved with breast cancer, such as BRCA1 and BRCA2, 14-3-3-sigma, and Her2, along with new and effective treatments. With new studies and discoveries, there may be a cure for this infamous disease as well as other cancers.

Acknowledgments

We would like to thank Stephanie Askeland, Angie Eakley, and Jen McGuire for the all of their peer advice and time. We would also like to thank, Dr. D for all of his energy and more importantly time in aiding in the understanding of breast cancer.

References

1. American Cancer Society. Retrieved March 17, 2002 from the World Wide Web: http:// www.cancer.org.
2. Coran, et al. (1999).. Robbins Pathological Basis of Disease. Pennsylvania: W.B. Saunders.
3. Breast Cancer History. Breast Cancer Society of Canada. Retrieved April 9, 2002 from the World wide Web: http://www.bcsc.ca.bcsc_htm/about/about_history.bc.html.
4. Contran, et al. (1999). Pathologic Basis of Disease. Penssylvania: W.B. Saunders.
5. The Genetic Basis of Breast Cancer Susceptibilty. Perfect Genes: Media Critics. Retrieved April 10, 2002 from the World Wide Web: http://www.web.Grinnell.edu/courses/sst/f01/sst395-01/PerfectGenes/CritLitRec/evanss.htm
6. Harari, Daniel, et al. Molecular Mechanisms Underlying ErbB2/HER2 action in breast cancer Oncogene 19, 6102-6114 (2000)
7. Venkitaraman, Ashok, et al. (2002) Cancer Susceptibility and the Functions of BRCA1 and BRCA2. Cell 108, 171-182
8. Venkitaraman, Ashok, et al. (1999) Breast Cancer Genes and DNA Repair. Science 286, 1100-1102
9. Scully, Ralph, et al. (2000) In search of the tumor-suppressor functions of BRCA1 and BRCA2. Nature 408, 429-432
10. Kachhap, S K, et al. (2001) Implication of BRCA1 gene in breast cancer. Indian Journal of Experimental Biology 39, 391-400
11.MacLachlan, Timothy, et al. (2000) BRCA1 Effects on the Cell Cycle and the DNA Damage Response Are Linked to Altered Gene Expression. Journal of Biological Chemistry 275, 2777-2785
12. Alberts, et al. (1998). Essential Cell Biology:An Introduction to the Molecular Biology of the Cell. New York: Garland.
13. Hermeking, et al. (1997) 14-3-3-sigma is a p53-regulated Inhibitor of G2/M Progression. Molecular Cell 1, 3-11.
14. Laronga, et al. (2000). Association of the cyclin-dependant Kinases and 14-3-3-sigma Negatively Regulates Cell Cycle Progression Journal of Biological Chemistry, 275 23106-23112
15. Chan, et al. (1999) 14-3-3-sigma is Required to Prevent Mitotic Catastrophe After DNA Damage. Nature, 401, 616-619.
16. Weaver, R. (1999) Molecular Biology. Massachusetts: McGraw Hill.
17. Ferguson, et al. (2000). High Frequency of Hypermethylation at the 14-3-3-sigma Locus Leads to Gene Silencing in Breast Cancer. Proceedings of the National Academy of Science, 97, 6049-6054.
18. Nass, et al. (2000) Aberrant Mathylation of the Estrogen Receptor and E-Cadnerin 5' CpG Islands Increase With Malignant Progression in Human Breast Cancer. Cancer Research, 60, 4346-4348.
19. Yuan, et al. (2001). Hypermethylation Leads to Silencing of the Syk Gene in Human Breast Cancer. Cancer Research, 61, 5558-5561.
21.HER-2/neu Oncoprotein: A New Target in the Treatment of Breas Cancerhttp://216.239.51.100/search?q=cache:I1OA8K qKC:www.labcorp.com/cme/CME_Her2_neu_Oncoprotein_Journal_Article_0447.pdf.
22. Andrechek, A, et al. (2000). Amplification of the neu/erbB-2 Oncogene in a Mouse Model of Mammary Tumorigenesis. Proc. Natl. Acad. Sci., 97 (7), 3777-3449.
23. Nelson, N. (2000). Can HER-2 Status Predict Response to Cancer Therapy? Journal of the National Cancer Institute, 92, 366-367.
24. Doherty, J., et al. (1999). The HER-2/neu Receptor Tyrosine Kinase Gene Encodes A Secreted Autoinhibitor. Proc.Natl.Sci., 96, 10869-10874
25. Casalini, P., et al. (2001) Role of p53 in HER-2-induced Proliferation or Apoptosis. The Journal of Biological Chemistry, 276 (15), 12449-12453.
26. Bajo, a., et al. (2002) Bombesin Antagonists Inhibit the Growth of MDA-MB-435 Estrogen-independent Breast
27.Gorman, Christine. (2002). "Rethinking Breast Cancer".Time, 159,50-58.