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The BRCA1 gene is mutated in approximately 40% of the breast cancers seen in hereditary breast cancer. The function of the BRCA1 gene is yet to be determined; however, it is thought to play a role in transcription regulation and/or DNA repair. The BE2 gene product was isolated because it was found to bind the BRCA1 protein. The BE2 gene has been localized to 11p15.5, loss of heterozygosity at this locus is seen in approximately 30% of spontaneous breast cancer. It is my content that BE2 is a novel tumor suppressor gene.

Functional assays are underway, which will determine if the BE2 cDNA suppresses in vitro and in vivo growth in breast cancer cell lines. The BE2 gene has been sequenced and Dr. Davis will use the sequence information to search for mutations in other breast cancer tissue. Dr. Davis will also characterize the BE2 gene product by assaying gene expression during different phases of the cell cycle. Immunohistochemistry will be used to determine the intra-cellular location of the BE2 gene product.

The identification of genetic markers that have prognostic significance is quite important. BE2 may prognostic significance. This information has the potential to augment medical therapy for breast cancer.

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FOREWORD

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The incidence of breast cancer continues to increase. It is estimated that by the year 2000, there will be one million new cases worldwide each year[1]. This is a disturbing trend because in the United States, breast cancer is already the third most common cancer accounting for 10% of all cancer deaths. The number of patients diagnosed with breast cancer and the number of deaths associated with this disease are sure to rise in the future.

As a result of past research efforts, the medical community has a better understanding of the pathologic process involved in the development of breast cancer. We are now able to detect breast cancer at earlier stages. Women are living longer with this diagnosis. The current surgical therapy has evolved as a result of ongoing research activity. Breast preservation surgery is more prevalent with a greater emphasis on the prevention of recurrent disease. As the scientific community learns more about carcinogenesis, the goals of therapy will change. The emphasis will not be the prevention of recurrent disease but the prevention of all disease.

The identification and isolation of the BRCA1 and BRCA2 tumor suppressor genes have affected breast cancer therapy. Mutations in these genes are seen most commonly in hereditary breast cancer families. Hereditary breast cancer accounts for 5-10% of breast cancer. BRCA1 mutations are present in 40-45% of the hereditary breast cancers and in 80% of ovarian cancers[2] A breast cancer family member with a mutation in the BRCA1 gene has a life time risk of 80-90% for developing breast cancer[3,4].

After establishing the linkage between BRCA1 mutations and hereditary breast cancer, much attention has been given to the characterization of BRCA1 functions. BRCA1 has been localized to 17q21. The N-terminus of the BRCA1 protein has a RING finger domain[2]. Proteins with this motif are involved in DNA recognition and/or protein-protein interactions. BRCA1 is thought to play a role in transcription regulation. The central portion of the BRCA1 protein has weak homology with the granin family[5]. Granins are acidic proteins that regulate the secretory pathways of cells. The BRCA1 protein may also play a role in cell-cell communication.

Studies on the intracellular location of the BRCA1 protein have helped in delineating its function. Chen et. al. state the BRCA1 codes for a 220kD protein that has been localized in the nucleus of normal cells and in the cytoplasm of breast cancer cells[6]. The nuclear localization of BRCA1 protein and the presence of a RING finger domain within this protein suggest that BRCA1 may play a role in the regulation of transcription. Therefore, the localization of the gene product in the cytosol may prevent its normal function and result in uncontrolled cellular proliferation.

Another method of determining the function of a gene product is to determine the binding characteristics. Work of this nature is being done in the laboratories of our collaborators, Drs. Anne Bowcock and Richard Bear[7]. The yeast two-hybrid system was utilized to identify proteins that bind to BRCA1 in vivo. The resultant BRCA1-associated-proteins were then screened using the mammalian two-hybrid assay. The mammalian two-hybrid system is a stringent assay and serves to eliminate false positives. Confirmation of binding to BRCA1 was shown using co-immunoprecipitation in mammalian cell lysate. In vitro assays also proved these proteins were associated with BRCA1.

The RING finger portion of the BRCA1 gene was used as a template in these experiments. This region is thought to have biologic significance and proteins that bind to the RING domain may help to delineate its function. This procedure lead to the isolation of the BRCA1 associated RING Domain gene (BARD1). This gene has been localized to 2q. This region of the genome has no known linkage to breast cancer and the function of the BARD1/BRCA1 heterodimer is unknown.

Another gene that has been isolated using the yeast two hybrid system is the BE2 gene. Unlike the BARD1 gene, the BE2 gene localizes to 11p15.5, a region known to have loss of heterozygosity (LOH) in breast cancer. Efforts are ongoing to identify the tumor suppressor in the region of 11p. BRCA1 mutations are seen in less than fifty percent of hereditary breast cancers and approximately 7% of sporadic tumors. Therefore, other genes are involved in the carcinogenic transformation of these tumors. If BE2 and BRCA1 contribute to the same normal functions, loss of either gene product may lead to the development of breast cancer. Therefore, the loss of BE2 may occur more frequently in sporadic tumors while BRCA1 dysfunction may account for many familial ones.

During my fellowship award period, I have worked on determining if BE2 is a novel tumor suppressor gene. I tested for functional activity by introduction of the gene into human breast cell lines. I will continue this work by looking for mutations within the gene using matched sets of normal and breast cancer tissues. Polyclonal and monoclonal antibodies will be used for intracellular localization of the BE2 protein. Finally, I will use biological assays to further characterize the role of BE2. I will also assay to see if there is functional overlap between the BE2 and the BRCA1 genes.

BE2 is an excellent candidate for a novel tumor suppressor gene because of its known association with BRCA1 and its localization to 11p15.5 of thehuman genome. This region has LOH in 20-30% of breast cancer. Dr. Laura Hink Reid performed the work done to localize BE2 to 11p15.5 in the Weissman lab. The deletion hybrid panel for 11p15.5 was screened for the presence of the BE2 gene. PCR amplification of this panel showed the BE2 gene to be located centromeric to D11S12 and telomeric to D11S20 (data not shown).

Dr. Anne Bowcock of the University of Texas supplied the cDNA sequence of the BE2 gene. The gene has two exons and encodes for a protein of 261 amino acids. I have performed PCR amplification across the known splice site and found the single intron to contain approximately 500 bp (data not shown). The BE2 gene is much smaller than BRCA1 and BRCA2 and this should facilitate the screening for mutations within the gene construct.

I have obtained the BE2 expression vectors from our collaborators. The vector is pCB6 that contains a CMV promoter and a neomycin resistance gene. BE2/pCB6 was constructed by inserting the BE2 coding sequence into the pCB6 vector. HA-BE2/pCB6 encodes for the BE2 gene product with an amino-terminal tag of 38 residues that includes the influenza hemaglutinin epitope. Monoclonal antibody 12CA5 (YPYDVPDYASL) recognizes the HA protein and can be used to identify expression of the HA transcript. The HA-BE2 gene product is also distinguishable by its mobility with polyacrylamide gel electrophoresis. (R. Baer, personal communication).

The vector constructs were transfected into three cell lines. The **MCF7** and **MDA 435** are well-characterized breast cancer cell lines. Both of these cells lines express wild type BRCA 1 (personal communication from R. Baer). The control cell line is **HeLa**. **Figure 1** is a western blot analysis of the parent cell lines utilized in these experiments. Endogenous expression of the 46-kiloDalton BE2 protein is observed in the **HeLa** and the **MDA 435** cell lines. The **MCF 7** breast cancer cell line is devoid of BE2 expression.

Polymerase chain reaction (PCR) will be used to confirm that the BE2 coding sequence has been incorporated into the different cell lines. PCR with primers for the neomycin resistance will be used to test the clones that were transfected with the vector alone.

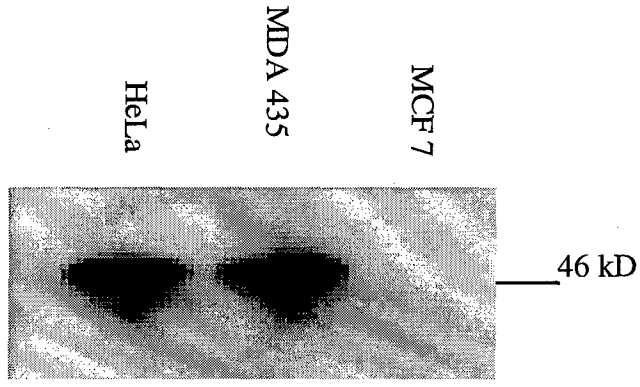

Figure 1. Western blot analysis of the parent cell lines. Monoclonal BE2 antibody was used to determine the expression pattern of the BE2 protein. The MDA435 and the HeLa cell lines show endogenous expression of the BE2 protien, while the MCF 7 is deviod of protein expression.

Figure 2 is a Western blot of the initial **MCF 7** transfectants. Detectable protein levels are visible in 2 of the 6 transfectants that contained the BE2 cDNA. No expression was observed in the clones transfected with the vector alone DNA. Preliminary results suggest that some clones derived from **HeLa** and **MDA 435** transfectants also express exogenous BE2 (data not shown). Therefore, a sufficient numbers of clones should be generated for the proposed in vivo and in vitro experiments.

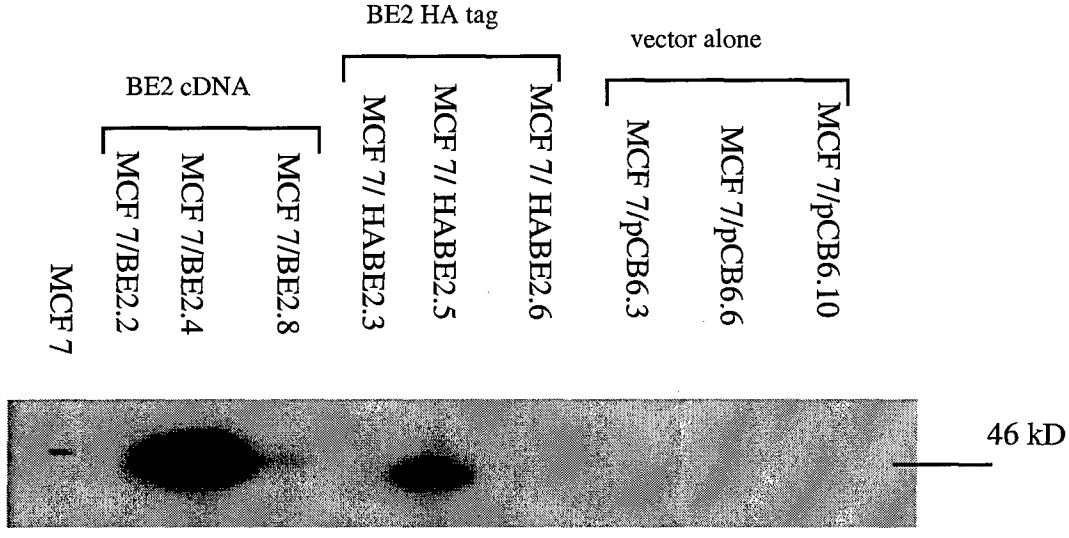

Figure 2. Western blot analysis of the MCF 7 transfectants. Monoclonal BE2 antibody was used to determine the expression pattern of the BE2 protein. Two transfectants (MCF 7/BE2.4 and MCF 7/HABE2.5) demonstrate expression of the BE2 protein while the parent, MCF 7, and the remainder of the transfectants are without detectable levels of the protein.

Co-immunoprecipitation assay was used to confirm that BE2 and BRCA1 interact. **Figure 3** is the result of this assay. The appropriate size band for the BE2 protein is seen in the transfected cell lines but not in the parent cell lines. There is an interaction between these two proteins. Further characterization of the BE2 protein will determine if its interaction with BRCA1 has clinical significance.

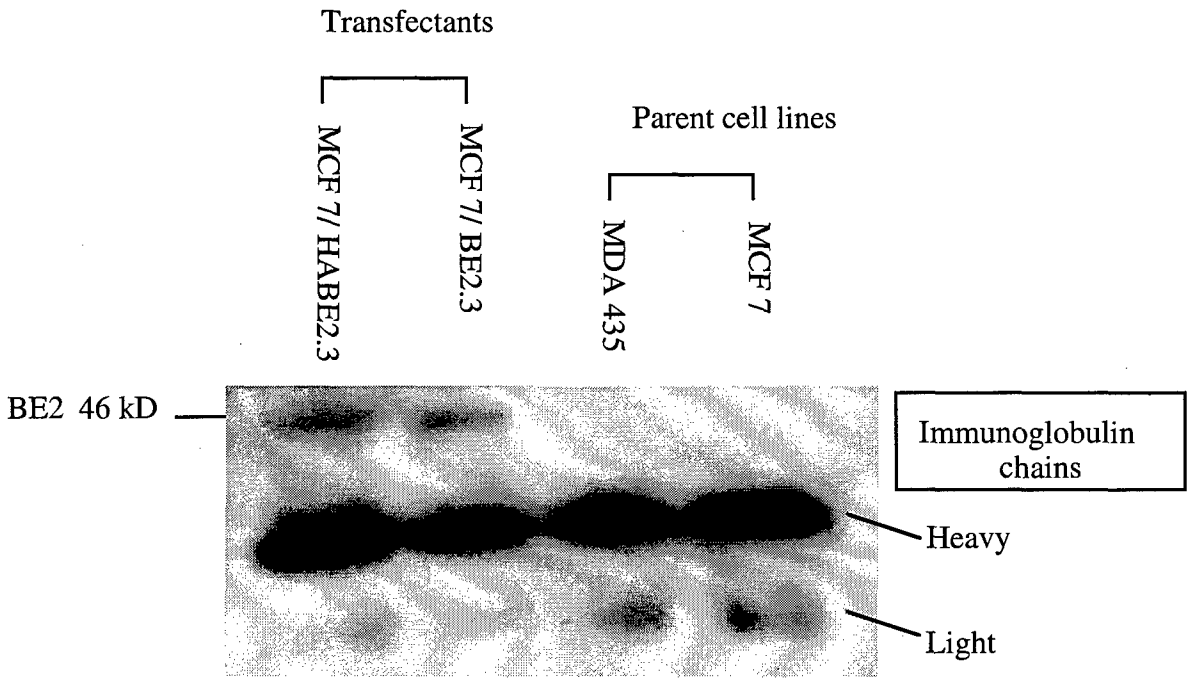

Figure 3. Mammalian cell lystaes were immunoprecipitated with a BRCA1 antibody. The aggregrates were then re-suspended and separted on a SDS-PAGE gel. Western blot analysis was then performed with a monoclonal antibody to the BE2 protein. BE2 is a 46 kiloDalton protein and its expression is demostrated in the transfected cell lines. This figure demonstrates that there is inded an interaction between BRCA1 and BE2.

Biologic assays which measure cellular proliferation, apoptosis and cell cycle distribution may prove helpful as I endeavor to characterize the BE2 gene. In vivo and in vitro growth assays will also be used to characterize the cell lines. These assays will be performed on the host cell line and the transfectants that contain the BE2 cDNA sequence. The in vivo growth assay is underway. The transfected cells have been injected into the mammary fat pads of nude mice. The mice will be followed for up to 12 week and tumor growth evaluated.

Summation

The characterization of BE2 gene will help to determine the function of its gene product. BE2 has known association with BRCA1. The exact nature of this association is yet to be determined. BRCA1 mutations are found in 40-45% of the breast cancers in hereditary breast cancer families. The BE2 gene may have a complementary or regulatory affect on the BRCA1 gene. Mutations in BE2 may therefore prevent the normal function of BRCA1. It is possible that mutations in the BE2 gene will be seen in a significant percentage of breast cancer family members with disease.

Alternatively, BE2 mutations could be seen in a higher percentage of sporadic breast cancers. Ninety percent of all breast cancers are the result of sporadic disease development. In these patients the identification of genetic markers which have prognostic significance is quite important. For example, breast cancer patients with mutations in ERBB2 fare worse than patients who do not have these mutations. It is possible that BE2 may have a similar prognostic significance. With such information, the clinician would improve his ability to predict disease. He would also be able to tailor the cancer therapy accordingly. This information has the potential to augment medical therapy for breast cancer. Work of this nature is important and needs to be done.

If I fail to find an association between the BE2 gene and the tumorigenic phenotype, I will use the known polymorphic markers that flank the BE2 gene and the markers in 11p15.5 to further localize the tumor suppressor gene in the region. Work of this nature has been performed in our lab.

Other candidate tumor suppressor genes are sure to be identified. Once isolated, I will use the above assays to determine their functional import. I have designed a system that will not only determine if the candidate gene functions as a tumor suppressor but also to characterize the manner in which tumor suppression is achieved.

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