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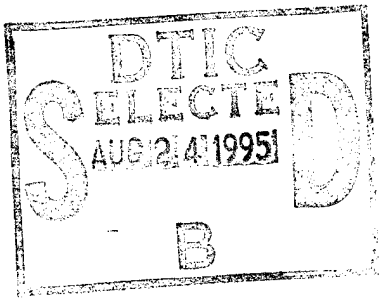

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Since erbB-2 failed to be a therapeutic response varibale for tamoxifen response, we have used gene trap U3lacZ to clone estrogen regulated molecules in breast cancer cells. Bcl-2 gene was identified as an estrogen inducible gene. A novel estrogen repressed gene was cloned. Their potential role as therapeutic response variable is being examined. NSABP B-11 was examined for erbB-2 expression. Statistical analysis of the B-11 is pending.

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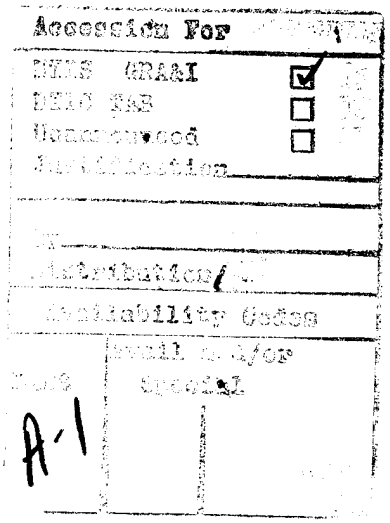

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Annual Report
June 5, 1995
Soonmyoung Paik, M.D.

Annual Report

Title: Custom tailoring of chemotherapy with erbB-2

Summary:
Our initial proposal was to examine erbB-2 as a therapeutic response variable that predicts the response to specific chemo or hormonal therpy in the treatment of breast cancer. Initial phase of the project involved the examination of 937 cases from the protocol B-14 of National Surgical Adjuvant Breast Project (NSABP). In this study, the value of erbB-2 as well as other markers such as S-phase fraction in predicting response to tamoxifen was examined. Since as stated in the last annual report erbB-2 failed to predcit response to tamoxifen, I have attempted to identify other markers that would have predictive value. Instead of examining other known markers, I elected to identify and clone new markers using retroviral gene trap. Using this approach, I have identified bcl-2 as an estrogen regulated molecule in breast cancer cells. In addition, a novel gene has been identified that is repressed upon estrogen treatment. Value of these new markers in the prediction of tamoxifen response will be examined in the future. Meanwhile, as a continuation of the originally proposed project on erbB-2, we have screened cases from NSABP B-11, in which patients were randomized to receive adriamycin vs. non-adriamycin adjuvant regimen. Unfortunately, due to the problems at the headquarters of NSABP, we have not yet completed the statistical analysis of these cases.

Introduction:
Treatment of breast cancer has been revolutionized through three important stages. First was the development of surgical techniques to remove breast cancer tissue which improved the survival of patients dramatically. Second stage was the use of systemic adjuvant therapy (tamoxifen for estrogen receptor positive tumors and chemotherapy for estrogen receptor negative tumors). The final revolution was the early detection through screening program. Even after these improvements in the diagnosis and management, we still did not obtain 100% cure of breast cancer. Thus, about 30% of early invasive breast cancer will still recur within 10 years after surgery and radiation. Additional systemic therapy will reduce this recurrence rate to about 20%. This modest benefit brings significant dilemma for clinical practice. Thus only small subset of patients actually benefit from systemic therapy. Focus of our lab has been to identify and test markers that predict response to systemic therapy. Our early attention to erbB-2 as a potential marker for tamoxifen response stems from in-vitro studies that demonstrated negative interaction between estrogen receptor signalling pathway and erbB-2 pathway. However, using samples from NSABP B-14, we failed to see any impact of erbB-2 in tamoxifen response. Thus we have changed our attention to other postential markers that will be useful as a predictor of tamoxifen response.
One of the important development in the recent year or two has been the identification apoptosis as a key process in cancer development and in chemotherapeutic action mechanism. Thus molecules involved in apoptotic pathway are potential candidates for therapeutic response variables. Estrogen is a key survival factor for breast epithelial cells. We have postulated that estrogen should inhibit molecules that are involved in apoptosis in order to maintain cell survival.

Thus we elected to clone genes that are regulated by estrogen.

Body:

Breast cancer cells and normal ductal epithelial cells which express the estrogen receptor (ER) undergo apoptosis upon estrogen withdrawal. This suggest the importance of estrogen in preventing apoptosis of estrogen receptor positive mammary ductal epithelial cells. For estrogen to suppress apoptosis it has to either repress molecular inducers of apoptosis and/or induce the expression of suppressors of apoptosis.

We have used the retroviral promoter trap U3lacZ to identify estrogen regulated genes from breast cancer cells so such genes could have an important role in the regulation of the apoptotic process in breast cancer cells. Clones in which the U3lacZ virus had integrated into genes which were either suppressed or induced by estrogen were identified. Beta-galatosidase reporter activity in 20 of 2000 clones mutated by U3lacZ were inducible by estrogen while 3 in 2000 were suppressed. Cloning and sequencing of 5'-flanking genomic DNA from one of the estrgoen inducible clones showed 100% identity with 5'-untranslated region of published bcl-2 cDNA sequence. LacZ reporter activity in this clone showed the classical regulation pattern of an estrogen regulated gene. Bcl-2 is believed to play a key role in the apoptotic process in hormone dependent organs including mammary gland. The induction of bcl-2 by estrogen in the breat cancer cell line MCF-7 was confirmed by western blot analysis. We have also seen a correlation between bcl-2 and ER expression in human breast cacner specimens which suggests that bcl-2 could be directly involved in apoptosis assiciated with estrogen withdrawal. Thus we identify bcl-2 as a candidate therapeutic response variable that predicts response to tamoxifen.

Cloning an sequencing of one of the estrogen repressed clones revealed a hereto undescribed gene that is down regulated by estrgogen.
The partial sequence of the gene is as follows:

1 agggctgtgg gcctgtatct tgtttgctcg tcatcctgtc agtcattttt ttctttccct
61 ttttttaaag acaaaatctg aacctagaaa caccgaagcc agagcaaaaa ctagatgcga
121 atccactatt tgtgcgaccc

Further characterization of this estrogen repressed gene is underway.

The strategy shown here could be useful in identifying genes regualted by hormones or growth factors. Clones in which genes inducible by estrogen are mutated by the U3lacZ retrovirus could be useful in screening anticancer drugs as the reporter gene lacZ faithfully follows the pattern of regulation of the endogenous genes.

Conclusions:

The conclusions from project year two can be summarized as follows:

1. erbB-2 does not predict response to tamoxifen.
2. Statistical analysis of B-11 to address whether erbB-2 predicts response to chemotherapy is pending.
3. Gene trap has identified bcl-2, an inhibitor of apoptosis, as an estrogen inducible gene. Thus bcl-2 is a candidate therapeutic response variable.
4. A nevel gene, which is repressed by estrogen in breast cancer cells, has been cloned using gene trap.