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INTRODUCTION

This project is testing the hypothesis that populations of women with significantly different demographic characteristics may not only have different incidences of breast cancers, but also different types of breast cancers. We are testing this hypothesis by examining gene expression profiles of breast cancers from two populations that differ apparently by only a single major variable: age. Specifically, we are measuring gene expression profiles by gene array technology and by immunohistochemistry in breast cancer tissues from young and elderly Korean women. Recently published studies have shown that this approach has great potential for classifying breast cancer at the molecular level. Korean breast cancers have been selected for this project because there is much less cultural and genetic diversity in the Korean population than in the North American population.

BODY

The overall progress of this project is summarized below:

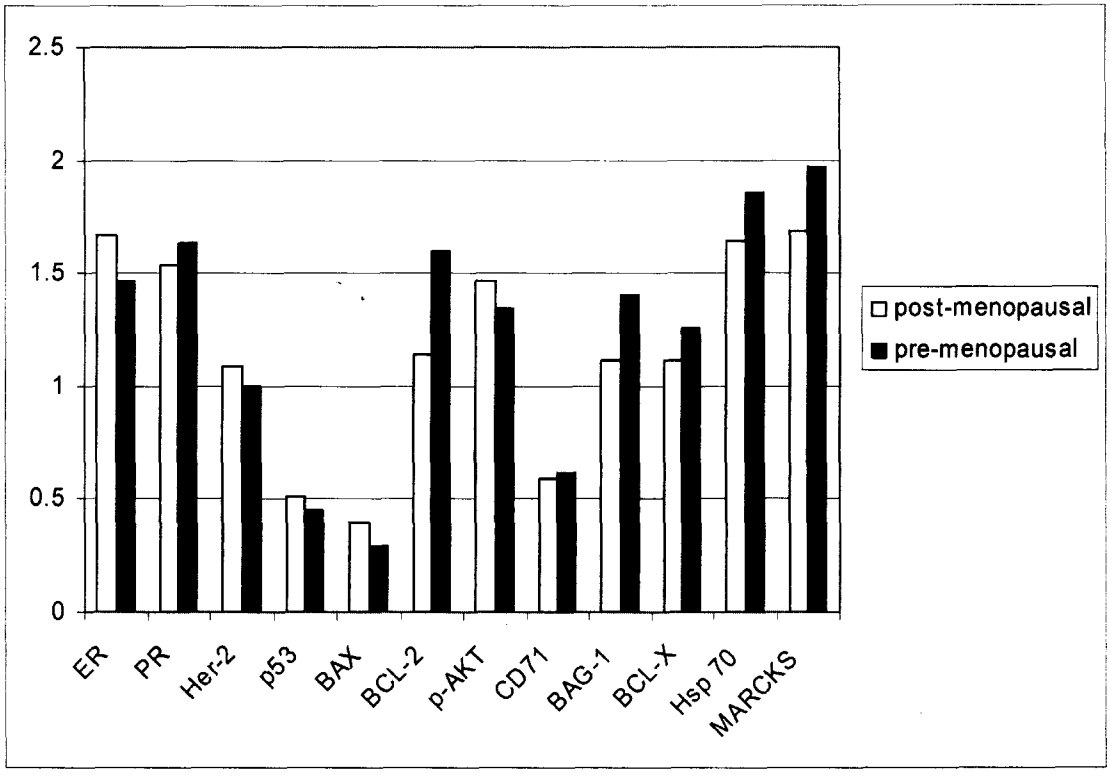
Gene array analysis to date fails to show age-specific patterns of gene expression in breast cancers (work conducted as part of Task 1).

We measured gene expression profiles in 22 young women (less than 45 years of age) and 21 elderly (over 65 years of age) women using cDNA arrays that represented 12,000 human genes. We performed both unsupervised hierarchical clustering and supervised class prediction models to determine whether the age differences in the patients determines the phenotype of breast cancer. The hierarchical clustering was performed with a variety of permutations using software developed at Stanford University (1), by adjusting cutoffs for use of genes in analysis (e.g., fold differences among samples). In none of these exploratory analyses were we able to find robust subclasses that were unique to either elderly or young patients.

With supervised classification methods, we assigned samples to one of two classes based on age of patient and then tested individual genes for differential expression across the two groups. Two methods were employed: a simple T statistic, and the SAM (Significance Analysis of Microarrays (2)), which also gives a measure of significance for each variable. By T test, 89 genes met a $p<0.01$ level of significance, but 120 genes (of 12,000) are expected to meet this level of significance by chance. Therefore, we have no reasonable certainty that any genes are differentially expressed (at a level greater than expected by chance) across the two groups. Similarly, SAM failed to show significant differential gene expression across the two groups.

We also conducted an analysis of 158 cases of Korean breast cancers represented on tissue arrays (3), using immunohistochemical techniques. In this set of cases, 75 of the cancers are from patients less than or equal to 45 years of age and 32 are from patients at least 56 years of age. Over 20 antibodies have been tested with these arrays, and none of the antigens show age-specific expression differences that are statistically significant. Data for some of these antibodies is summarized in figure 1 below.

Figure 1: Mean immunohistochemical staining scores for 12 antibodies that show highly variable expression across different breast cancers. No significant age-specific differences were noted.



KEY RESEARCH ACCOMPLISHMENTS

- Microarray studies completed
- Supervised and Unsupervised data analysis conducted
- Tissue microarray studies conducted

REPORTABLE OUTCOMES

Work using the tissue microarrays that were developed with this funding was presented as a poster and platform presentation at the DOD Era of Hope Meeting in Orlando, Florida, September 2002. Specifically, these tissue microarrays were being used to investigate role of apoptosis protein expression in predicting outcome for these patients. A manuscript describing the profile of apoptosis proteins related to outcome in breast cancer is in revision.

CONCLUSIONS

We have found that the gene expression profiles of breast cancers in Korean women do not show significant age-related differences. Unfortunately, these results do not warrant

additional testing of the hypothesis as originally proposed. However, this project has generated an important tissue resource that is being used to study markers that predict breast cancer outcome. In particular, we have important findings regarding the expression of apoptosis proteins and clinical outcome in Korean breast cancer patients.

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