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ABSTRACT

The 5-year survival rate for BC among US women has increased from 75% during 1974-76 to 85% during 1989-95. Despite such marked improvement, BC is still the leading cause of cancer mortality among women 20 – 59 years of age and the second leading cause of cancer mortality among all women. Disease-free survival after BC treatment is likely predicted by both tumor characteristics and host factors. The clinical and pathologic parameters that have been shown to influence disease prognosis include tumor size, nodal involvement, tumor state, grade, hormone receptor status, mitotic index, expression of multi-drug resistance proteins, p53 status, and HER-2/neu status. Meanwhile, only a few host factors have been identified that impact disease-free or overall survival, particularly those that a patient may engage in to modify or help clinicians to tailor effective and efficient treatment strategy. This proposed study focuses on one-carbon metabolism, a key process for DNA methylation and DNA synthesis. One-carbon metabolism is crucial of BC prognosis because it not only provides methyl group for regulating expression of genes that have prognostic values (e.g. *ER, PR, BRCA1,* etc.) but also is a primary target for treatment of the disease (e.g. 5-FU, methotrexate, etc.). We propose to utilize the resources of the Long Island Breast Cancer Study Project, a large population-based study consisting of ~1500 BC cases and ~1500 controls. We will examine the dietary intake of one-carbon-related micronutrients/compounds (e.g. folate, methionine, chioline, B vitamins, alcohol, etc) in relation to disease-free and overall survival of BC *via* the mechanism of promoter hypermethylation (presumably silencing) of the ER, PR, and BRCA1 genes. We will also examine whether functional polymorphisms in one-carbon metabolism may influence survival of BC, either through modifying the efficacy of chemotherapeutic drugs or influencing methylation of prognosis-related genes. Results from this study would help clarify mechanisms of disease progression as well as contribute to the design of a more efficient (genetically tailored) treatment strategy.

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INTRODUCTION

The 5-year survival rate for BC among US women has increased from 75% during 1974-76 to 85% during 1989-95[1]. Despite such marked improvement, BC is still the leading cause of cancer mortality among women 20 – 59 years of age and the second leading cause of cancer mortality among all women. Disease-free survival after BC treatment is likely predicted by both tumor characteristics and host factors. The clinical and pathologic parameters that have been shown to influence disease prognosis include tumor size, nodal involvement, tumor state, grade, hormone receptor status, mitotic index, expression of multi-drug resistance proteins, p53 status, and HER-2/neu status. Meanwhile, only a few host factors have been identified that impact disease-free or overall survival, particularly those that a patient may engage in to modify or help clinicians to tailor effective and efficient treatment strategy. This proposed study focuses on one-carbon metabolism, a key process for DNA methylation and DNA synthesis. One-carbon metabolism is crucial of BC prognosis because it not only provides methyl group for regulating expression of genes that have prognostic values (e.g. *ER, PR, BRCA1,* etc.) but also is a primary target for treatment of the disease (e.g. 5-FU, methotrexate, etc.). We propose to utilize the resources of the Long Island Breast Cancer Study Project, a large population-based study consisting of ~1500 BC cases and ~1500 controls. We will examine the dietary intake of one-carbon-related micronutrients/compounds (e.g. folate, methionine, chioline, B vitamins, alcohol, etc) in relation to disease-free and overall survival of BC *via* the mechanism of promoter hypermethylation (presumably silencing) of the ER, PR, and BRCA1 genes. We will also examine whether functional polymorphisms in one-carbon metabolism may influence survival of BC, either through modifying the efficacy of chemotherapeutic drugs or influencing methylation of prognosis-related genes. Results from this study would help clarify mechanisms of disease progression as well as contribute to the design of a more efficient (genetically tailored) treatment strategy.

BODY

Task 1. **To genotype polymorphisms in one-carbon-metabolizing genes on 1087 BC cases (Months 1- 24)**

a. Prepare aliquotes of all DNA sample to be sent to BioServe for genotyping.

Genomic DNA has been isolated from all 1087 BC cases (as well as 1150 controls). They have been sent to BioServe to be plated.

b. Set up and validate the genotyping assay for all SNPs.

Sample plates have been assembled at BioServe. Genotyping assay has been set up and optimized for the following three polymorphisms:

MTR 2756A>G: rs1805087
BHMT 742G>A; rs3733890
RFC1 80G>A, rs1051266

c. Ascertain proposed genotypes by a MALDI-TOF-based method.
d. QC check and data entry.

Genotyping has been completed for the 3 polymorphisms mentioned above. QC check has been completed; the error rate is 0%, 0% and 99% for MTR, BHMT and RFC1, respectively. The genotype distribution for these polymorphisms are listed in Table 1. All genotype distributions at these three loci were in agreement with Hardy-Weinberg Equilibrium. In addition, genotyping for the MTHFR 677C>T, 1298A>G and TSTR polymorphisms have been completed by the support of the DOD grant (DAMD17-00-1-0345)

Table 1. Genotype distribution of MTR, BHMT and RFC1 polymorphisms among the cases of LIBCSP deterimined by MALDI-TOF-based method.

		N	%
MTR 2756A>G			
	AA	666	66.8
	AG	296	29.7
	GG	35	3.5
BHMT 742G>A			
	AA	498.	49.7
	AG	442	42.1
	GG	83	8.3
RFC1 80G>A			
	GG	265	27.6
	GA	486	50.7
	AA	208	21.7

Task 2. Determine the promoter methylation patterns on *ER, PR,* and *BRCA1* genes from ~960 BC tissues (Months 1-30)

a. DNA extraction from ~960 tumor blocks.

We have isolated genomic DNA from 879 tumor blocks by microdissecting two 10 micron slides. Quantification of DNA was not determined because the concentration is too low for UV or fluorescence methods.

b. Set up and validate methylation assay on *ER, PR* and *BRCA1* genes using a real-time quantitative methylation-specific PCR method.

We have also established the MethyLight assay for *BRCA1* using a positive control cell line UACC3199 developed by Dr. Bernie Futscher, University of Arizona, and MCF7 as a negative control cell line. Primers and probes for *BRCA1* as well as for *beta actin* (ACTB), used as a reference set to normalize for input DNA, were based on Muller *et al.*[2] Since the last submission, this assay has been applied to bisulfite-treated DNA isolated from 20 LIBCSP tumor DNAs that were successfully amplified for all *p53* exons. All samples could be amplified using the *ACTB* primers and probe. Figure 1 gives representative data on the positive control cell line (blue) as well one of the tumors (red) for *ACTB*. Three of the 20 samples were positive for *BRCA1* methylation. Representative data for the positive control cell line (green) and one of the positive tumors (black) for *BRCA1* is also illustrated in the figure. After running the MethyLight assay, DNA from the positive wells was run on a gel confirming the presence of the appropriate molecular weight band.

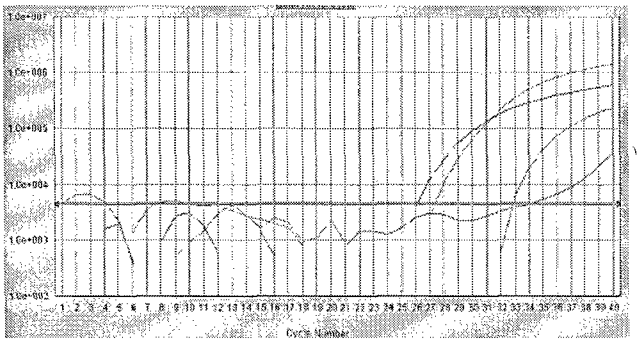


Figure 1: Representative MethyLight analysis of LIBCSP tumor DNA for ACTB and BRCA1

c. Ascertain methylation patterns of *ER, PR*, and *BRCA1* from 960 tumor tissues.
d. Data entry.

In progress.

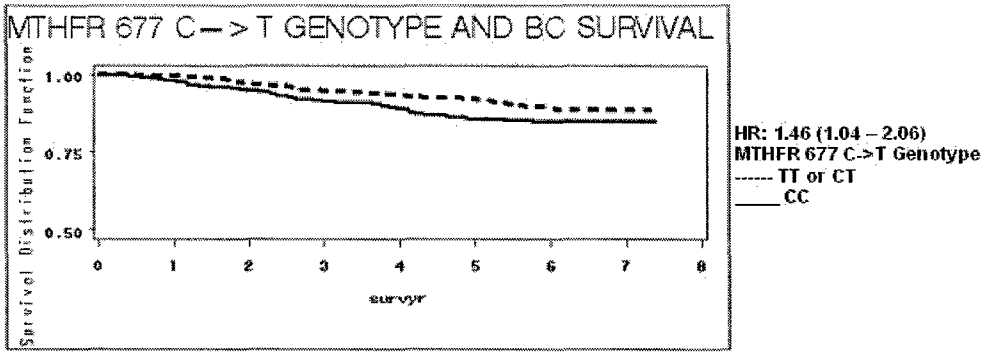
Task 3. **Data analyses (Months 25-36)**

a. Study associations of dietary methyl content and overall survival.

We are now in the process of converting dietary intakes from post-diagnose questionnaires into nutrition scores. This task will be carried out at IMS.

b. Study associations of one-carbon polymorphisms and overall survival.

We have some preliminary data on the association between the MTHFR677C>T polymorphism and 5-year overall survival. In this preliminary analysis, cases with CT or TT genotype had a poor survival rate compared to the CC wildtypes. The HR is 1.46 (95%CI 1.04-2.06).



Overall survival of the BC cases in the LIBCSP affected by MTHFR 677 C->T genotypes: CC versus TT or CT

c. Study associations of one-carbon metabolism (diet and polymorphism) and methylation patterns of *ER, PR* and *BRCA1*.

In progress.

d. Study associations of methylation pattern and overall survival.
e. Study survival relationship by treatment regimen (i.e. chemotherapy vs. no chemotherapy).
f. Manuscript preparation.

These three tasks will be carried out in the future.

KEY RESEARCH ACCOMPLISHMENTS

1. We have completed genotyping for MTHFR, TS, MTR, BHMT and RFC1 polymorphisms from the cases of the LIBCSP.
2. We have isolated genomic DNA from 879 tumor blocks. They are ready for the methylation analyses.
3. We have set up the MethyLight assay for BRCA1 promoter methylation.
4. Our preliminary analysis indicates that the MTHFR 677C>T polymorphism may be associated with breast cancer survival.

REPORTABLE OUTCOMES

None

APPENDICES

None