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14. 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.

15. SUBJECT TERMS

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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)

Genotyping has been completed for the 9 polymorphisms listed in **Table 2**. About 10% samples were duplicate samples for quality control purpose; the rate of concordance between the duplicate samples were >99%. 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, tested by the goodness-of-fit test.

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 all tumor blocks (n=916) by microdissecting two 10 micron slides. Quantification of DNA was not determined because the concentration is too low for UV or fluorescence methods. We have finished bisulfid treatment of all cancer DNA samples (finished modifying all samples).

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

We have established the MethyLight assay for all three loci proposed in the study, i.e. P16, BRCA1 and ER. Figure 1 is an example for the BRCA1 locus.

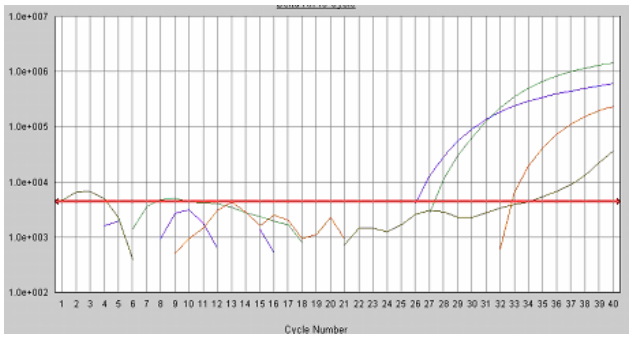

Figure 1: Representative MethyLight analysis of LIBCSP tumor DNA for ACTB and BRCA1

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

We are in the process of determining the methylation status for the breast cancer samples. At present, 36, 83 and 69 cases have been assayed for the p16, BRCA1 and ER, respectively. We detected 8/36,36/83,6/69 promoter hypermethylation for the respective loci.

d. Data entry.

In progress.

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

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

We have finished converting dietary intakes from post-diagnose questionnaires into nutrition scores. This task was carried out at IMS.

Descriptive statistical analysis of the survival distribution of the patients was performed.
Overall and sub-category results were summarized in **Table 1**. The association between overall survival time and time to disease progression with genotypes was estimated using the Kaplan-Meier method and assessed using the log-rank test. Median survival time was calculated. We employed Cox proportional hazards models (SAS) for failure-time data to assess associations of categories of genotypes with time to breast cancer death or all-cause mortality and obtained breast cancer specific or overall survival results Hazard ratios and 95% CIs were estimated using univariate or multivariate Cox proportional hazards models. We considered the following known or potential clinical prognostic factors in the multivariate model: age, menopausal status(pre- and post-), ER/PR status(four categories: ER+/PR+, ER+/PR-,ER-/PR+, ER-/PR-) and cancer stage (in situ or invasive).

Table 1 Descriptive Analysis

	Sub-graoup	**No. of subject**	**No. of censored**	**No. of dead**	**Mean time for death, months (range)**	**Median time for death, months**	**Mean age at diagnosis, yearsrange)**	**5-year survival rate**
Overall		1508	1310	198				
BC specific				114	37.8(2.7,72.8)	33.6	57.6(29.7,98.1)	0.95
Menopau-sal status	Pre-	472	431	41	40.6(11.7,72.8)	39.6	45.5(27.5,66.5)	0.91
	Post-	1006	935	71	36.6(2.7,71.6)	32.8	65.3(25,98)	0.88
ER/PR status	-/-	212	160	52	31.5(4.9,70.9)	28.9	56.6(25.1,93.6)	0.77
	-/+	52	44	8	35.8(2.7,65.2)	38.3	55.2(35.8,81.8)	0.89
	+/-	143	115	28	39.9(5.9,72.8)	44.3	64.6(29.7,89.7)	0.83

	+/+	583	522	61	40.6(6.4,71.6)	38.2	59.7(28.2,98.1)	0.91
Cancer type	In situ	235	225	10	42(14.3,65.2)	44.0	56.4(31.3,88.6)	0.97
	Invasive	1273	1085	189	37.4(2.7,72.8)	34.4	59.3(25,98)	0.87

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

The associations of 9 polymorphisms of the one-carbon metabolizing genes with overall survival were summarized in **Table 2.** Overall, the these polymorphisms were not associated with the overall survival. However, there are several heterozygous genotypes showing statistically significant better suvival compared to the reference genotypes:

MTHFR(C677T): HR 0.58(0.39-0.85)
BHMT: HR 0.63(0.43-0.93)
MTRR: HR 0.64(0.43-0.96)

Table 2 Associations of polymorphisms of the one-carbon metabolizing genes with overall survival

Gene	Genotype	No. failed	No. censored	No. censored (%)	Mean Survival Time (Years)	Hazard Ratio*	95% CI
MTHFR* ***(C677T)***	**CC**	60	337	337(85%)	**5.31**	Ref	--
	CT	43	433	433(91%)	**5.85**	**0.58**	**0.39 -- 0.85**
	TT	28	161	161(85%)	**5.24**	0.99	0.63 - 1.55
TSTR*	**3R/3R**	43	265	265(86%)	**5.66**	Ref	--
	3R/2R	54	449	449(89%)	**5.65**	0.76	0.51 - 1.13
	2R/2R	30	197	197(87%)	**5.42**	0.89	0.56 - 1.43
BHMT* ***(G742A)***	**GG**	73	436	436(86%)	**5.67**	Ref	--
	AG	40	403	403(91%)	**5.17**	**0.63**	**0.43 - 0.93**
	AA	17	92	92(84%)	**5.48**	1.09	0.64 - 1.85
DHRF* ***(19bp del)***	**+/+**	45	285	285(86%)	**5.64**	Ref	--
	+/-	59	449	449(88%)	**5.46**	0.88	0.54 - 1.44
	-/-	25	184	184(88%)	**5.59**	0.87	0.59 - 1.29
MTRR* ***(A66G)***	**GG**	40	222	222(85%)	**5.51**	Ref	--
	AG	58	493	493(89%)	**5.63**	**0.64**	**0.43 - 0.96**
	AA	31	200	200(87%)	**5.67**	0.84	0.53 - 1.34
MTHFR* ***(A1208C)***	**CC**	68	485	485(88%)	**5.71**	Ref	--
	AC	48	362	362(88%)	**5.63**	0.97	0.67 - 1.41
	AA	13	74	74(85%)	**4.9**	1.31	0.72 - 2.4
RFC1* ***(A80G)***	**GG**	35	253	253(88%)	**5.32**	Ref	--
	AG	61	468	468(88%)	**5.74**	0.89	0.59 - 1.35
	AA	35	213	213(86%)	**5.38**	1.2	0.75 - 1.9
MTR* ***(A2756G)***	**GG**	96	609	609(86%)	**5.57**	Ref	--
	AG	30	282	282(90%)	**5.78**	0.69	0.46 - 1.04
	AA	3	33	33(92%)	**5.51**	0.52	0.17 - 1.65
cSHMT*	**CC**	71	455	86.5	**5.65**	Ref	--

(C1420T)	**CT**	53	415	88.7	**5.47**	0.77	0.54-1.1
	TT	11	88	88.9	**5.14**	0.78	0.41-1.47

* Adjusted for age

The associations of these 9 polymorphisms of the one-carbon metabolizing genes with breast cancer specific survival were summarized in **Table 3.**
Overall, the these polymorphisms were not associated with breast cancer specific survival. However, there are several heterozygous genotypes showing statistically significant better suvival compared to the reference genotypes:
MTHFR(C677T): HR 0.56(0.33-0.94)
BHMT: HR 0.49(0.28-0.86)
MTRR: HR 0.55(0.32-0.94)

Table 3 Associations of polymorphisms of the one-carbon metabolizing genes with breast cancer specific survival

Gene	Genotype	No. failed	No. censored	No. censored (%)	Mean Survival Time (Years)	Hazard Ratio*	95% CI
MTHFR*	**CC**	34	363	91.4	**5.15**	Ref	--
(C677T)	**CT**	24	452	95	**5.95**	**0.56**	**0.33-0.94**
	TT	14	175	92.6	**5.36**	0.86	0.46-1.59
TSTR*	**3R/3R**	22	286	92.9	**5.85**	Ref	--
	3R/2R	30	473	94	**5.8**	0.83	0.48-1.44
	2R/2R	18	209	92.1	**5.16**	1.14	0.61-2.13
BHMT*	**GG**	40	469	92.1	**5.83**	Ref	--
(G742A)	**AG**	18	425	95.9	**5.29**	**0.49**	**0.28-0.86**
	AA	13	96	88.1	**5.63**	1.54	0.82-2.87
DHRF*	**+/+**	26	304	92.1	**5.82**	Ref	--
(19bp del)	**+/-**	30	478	94.1	**5.25**	0.74	0.44-1.25
	-/-	14	195	93.3	**5.74**	0.82	0.43-1.58
MTRR*	**GG**	24	238	90.8	**5.66**	Ref	--
(A66G)	**AG**	28	523	94.9	**5.77**	**0.55**	**0.32-0.94**
	AA	19	212	91.8	**5.84**	0.88	0.48-1.6
MTHFR*	**CC**	37	516	93.3	**5.86**	Ref	--
(A1208C)	**AC**	25	385	93.9	**5.79**	0.9	0.54-1.49
	AA	9	78	89.7	**5**	1.57	0.76-3.26
RFC1*	**GG**	19	269	93.4	**5.39**	Ref	--
(A80G)	**AG**	34	495	93.6	**5.89**	0.96	0.55-1.68
	AA	19	229	92.3	**5.16**	1.16	0.62-2.2
MTR*	**GG**	47	658	93.3	**5.77**	Ref	--
(A2756G)	**AG**	23	289	92.6	**5.84**	1.08	0.65-1.77
	AA	0	36	100	**.**	.	.
						.	.
cSHMT*	**CC**	41	485	92.2	**5.82**	Ref	
(C1420T)	**CT**	29	439	93.8	**5.4**	0.78	0.48-1.26

	TT	5	94	94.9	**5.3**	0.64	0.25-1.63

* Adjusted for age

We are in the process of in depth analyses, including model building and multivariate analyses.

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 9 polymorphisms from the cases of the LIBCSP.
2. We have isolated genomic DNA from all available tumor blocks (n=916). They have all been bisulfite treated and ready for the methylation assays.
3. We have set up the MethyLight assay for all three loci proposed in the study, i.e. p16, BRCA1, and ER. The methylation analyses are well underway.

CONCLUSIONS

Our preliminary analysis indicates that several polymorphisms may be associated with breast cancer survival.

REPORTABLE OUTCOMES

None

APPENDICES

None