

Composite Biomarker for Breast Cancer

For non-invasive screening, diagnosis, and prognosis

Abstract

Based on a review of 1,787 publications, 345 genes were identified as hypermethylated in the tumors of breast cancer patients when compared to normal tissues. Of these 345, the expression level of 221 genes were found in two independent studies. The first study assessed the expression of these genes in 18 histologically normal breast epithelium from ER- or ER+ breast cancer patients and were then compared to healthy tissues from 18 patients who received breast reduction mammoplasty. The second study assessed the same 221 genes in 14 autologous normal tissues from breast cancer patients as compared to 15 normal tissues taken from reduction mammoplasty.

The data from the two studies was pooled together and a computational method was used to analyze the methylation in serum of all 221 genes in the 32 breast cancer patients and the 33 cancer-free subjects. Of these 221 genes, 19 genes show a high potential as screening markers, even in the presence of noise.

Patients

The expression level of 221 genes were found in two independent studies:

- ▲ In 18 autologous histologically normal breast epithelium from ER- or ER+ breast cancer patients compared to 18 reduction mammoplasty tissues from normal subjects (Graham, et al., *British Journal of Cancer* [2010] 102, 1284–1293).
- ▲ In 14 tumor-adjacent, histologically normal breast TDLUs microdissected from patients with untreated ER+ breast cancers (cancer normal [CN]) as compared to 15 healthy tissues from reduction mammoplasty subjects (Anusri Tripathi, et al., *International Journal of Cancer* [2008] 122, 1557–1566).

Table 1 and Table 1b on the following page show the patients' characteristics.

Advantages

- ▲ The identified genes' methylation pattern can be used in simple tests for screening, diagnosis, and prognosis of breast cancer.
- ▲ The combined methylation patterns provide 97% sensitivity and 100% specificity for breast cancer diagnosis.
- ▲ The assessment of methylation is a very stable procedure that is not influenced by experimental parameters, making this an efficient test in any clinical laboratory.
- ▲ Tests can be done efficiently using serum and other body fluids, which is more acceptable to patients and easier to incorporate into both primary care and hospital-based healthcare settings.
- ▲ This test could easily and at low cost be used in national screening programs.
- ▲ The test may decrease the high incidence of mortality by detecting breast cancer at earlier stage.

Table 1 (left) and Table 1b (right): Patient Characteristics for Breast Cancer

Samples	Factors		Title
	Specimen	Disease State	
GSM512539	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 1
GSM512540	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 2
GSM512541	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 3
GSM512542	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 4
GSM512543	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 5
GSM512544	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 6
GSM512545	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 7
GSM512546	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 8
GSM512547	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 9
GSM512548	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 10
GSM512549	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 11
GSM512550	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 12
GSM512551	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 13
GSM512552	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 14
GSM512553	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 15
GSM512554	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 16
GSM512555	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 17
GSM512556	reduction mammoplasty	control	reduction mammoplasty breast epithelium sample 18
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GSM512566	ER- breast cancer	breast cancer	histologically normal breast epithelium from ER-breast cancer patient sample 1
GSM512567	ER- breast cancer	breast cancer	histologically normal breast epithelium from ER-breast cancer patient sample 2
GSM512568	ER- breast cancer	breast cancer	histologically normal breast epithelium from ER-breast cancer patient sample 3
GSM512569	ER- breast cancer	breast cancer	histologically normal breast epithelium from ER-breast cancer patient sample 4
GSM512570	ER- breast cancer	breast cancer	histologically normal breast epithelium from ER-breast cancer patient sample 5
GSM512571	ER- breast cancer	breast cancer	histologically normal breast epithelium from ER-breast cancer patient sample 6
GSM512572	ER- breast cancer	breast cancer	histologically normal breast epithelium from ER-breast cancer patient sample 7
GSM512573	ER- breast cancer	breast cancer	histologically normal breast epithelium from ER-breast cancer patient sample 8
GSM512574	ER- breast cancer	breast cancer	histologically normal breast epithelium from ER-breast cancer patient sample 9
GSM512557	ER+ breast cancer	breast cancer	histologically normal breast epithelium from ER+ breast cancer patient sample 1
GSM512558	ER+ breast cancer	breast cancer	histologically normal breast epithelium from ER+ breast cancer patient sample 2
GSM512559	ER+ breast cancer	breast cancer	histologically normal breast epithelium from ER+ breast cancer patient sample 3
GSM512560	ER+ breast cancer	breast cancer	histologically normal breast epithelium from ER+ breast cancer patient sample 4
GSM512561	ER+ breast cancer	breast cancer	histologically normal breast epithelium from ER+ breast cancer patient sample 5
GSM512562	ER+ breast cancer	breast cancer	histologically normal breast epithelium from ER+ breast cancer patient sample 6
GSM512563	ER+ breast cancer	breast cancer	histologically normal breast epithelium from ER+ breast cancer patient sample 7
GSM512564	ER+ breast cancer	breast cancer	histologically normal breast epithelium from ER+ breast cancer patient sample 8
GSM512565	ER+ breast cancer	breast cancer	histologically normal breast epithelium from ER+ breast cancer patient sample 9

Samples	Disease State	Title
GSM241999	normal	reduction mammoplasty patient 288
GSM242000	normal	reduction mammoplasty patient 278
GSM242001	normal	reduction mammoplasty patient 309
GSM242002	normal	reduction mammoplasty patient 310
GSM242003	normal	reduction mammoplasty patient 314
GSM242004	normal	reduction mammoplasty patient 334
GSM242005	normal	reduction mammoplasty patient 340
GSM242006	normal	reduction mammoplasty patient 350
GSM242007	normal	reduction mammoplasty patient 357
GSM242008	normal	reduction mammoplasty patient 347
GSM242009	normal	reduction mammoplasty patient 352
GSM242010	normal	reduction mammoplasty patient 361
GSM242011	normal	reduction mammoplasty patient 328
GSM242012	normal	reduction mammoplasty patient 360
GSM242013	normal	reduction mammoplasty patient 368
GSM242014	cancer	breast cancer patient 251
GSM242015	cancer	breast cancer patient 318
GSM242016	cancer	breast cancer patient 320
GSM242017	cancer	breast cancer patient 359
GSM242018	cancer	breast cancer patient 297B
GSM242019	cancer	breast cancer patient 316
GSM242020	cancer	breast cancer patient 304B
GSM242021	cancer	breast cancer patient 351
GSM242022	cancer	breast cancer patient 248
GSM242023	cancer	breast cancer patient 226
GSM242024	cancer	breast cancer patient 258
GSM242025	cancer	breast cancer patient 274
GSM242026	cancer	breast cancer patient 232
GSM242027	cancer	breast cancer patient 237

Results

The methylation profile of several combinations of these 19 genes enables researchers to discriminate breast cancer (BC) from healthy individuals, with high sensitivity and specificity. Table 2 (below) shows the methylation status of the 32 patients, where "TRUE" is defined as "the methylation value is greater or equal to the highest methylation value in normal individuals plus an error margin" for an available dataset. The error is represented by a margin of 5% over the highest methylation value in normal patients. The error margin is defined as 0.05 times the difference between the full methylation value (100% methylation) and the highest methylation value in controls.

The table below demonstrates how the combination of these genes has the potential to identify patients with BC with a specificity of 100% and a sensitivity of 97%.

Table 2: The Data: Predicted methylation values from 32 breast cancer patients. **Values shown:** Calls for 32 BC patients defined as "TRUE" (the serum predicted methylation value is above the 5% threshold) or defined as "FALSE" (the serum predicted methylation value is below the threshold). The combination of 19 biomarkers described in the table affords 97% sensitivity and 100% specificity when asking at least one "TRUE" call to be diagnosed as having BC.

		97% Sensitivity and 100% Specificity																			
5% cutoff	Sp	BioM1	BioM2	BioM3	BioM4	BioM5	BioM6	BioM7	BioM8	BioM9	BioM10	BioM11	BioM12	BioM13	BioM14	BioM15	BioM16	BioM17	BioM18	BioM19	
Autologous Normal Tissue (breast cancer patients)	1	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	
	2	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	TRUE	FALSE	FALSE	TRUE
	3	FALSE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE
	4	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE
	5	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
	6	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE
	7	FALSE	FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
	8	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE
	9	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
	10	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE
	11	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE
	12	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE
	13	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE
	14	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	TRUE	TRUE
Autologous to ER+ breast cancer	15	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	
	16	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	
	17	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	
	18	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	
	19	FALSE	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	
	20	FALSE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	
	21	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	
	22	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	TRUE	FALSE	
	23	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	
	24	TRUE	TRUE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	
Autologous to ER- breast cancer	25	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	
	26	TRUE	TRUE	FALSE	TRUE	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	
	27	TRUE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	
	28	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	
	29	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	FALSE	FALSE	
	30	FALSE	TRUE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	
	31	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	
	32	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	

For More Information

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