



Supplementary Material

DNA Methylation Biomarkers for Diagnosis of Breast Cancer: Focus on *FGFR1* and *FGF2*

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Supplementary Table I. Summary of DNA methylation studies in breast cancer.

Study title	Accession number	Platform	No. of samples	Genes/CpG sites studied
Study 1: DNA Methylation Profiling in Carolina Breast Cancer Study	GSE32393	Illumina Golden Gate Methylation Cancer Panel I	517 breast tumors, 9 normal breast tissues	935 CpG sites
Study 2: DNA Hypermethylation in Breast Cancer: Breast Cancer Tissue vs Normal Tissue	GSE20713	Agilent 60-k CpG2	10 tumor, 10 normal breast tissues	61,982 CpGs across 4,162 genes
Study 3: Breast Cancer Methylomes Establish an Epigenomic Foundation for Metastasis	GSE31879	Illumina Human Methylation 27 BeadChip	39 breast cancer primary samples	Large number of genes
Study 4: Analysis of Promoter Methylation in Breast Cancer	GSE32394	Illumina Human Methylation 27 BeadChip	47 primary breast tumors, 10 normal tissues	N/A
Study 5: Luminal B Breast Cancer Subtype Displays a Dichotomic Epigenetic Pattern	GSE31979	Illumina HumanMethylation 27 BeadChip	29 luminal subtype, 8 control tissues	N/A
Study 6: Epigenetic Portraits of Human Breast Cancer	GSE20711	Illumina Human Methylation 27 BeadChip	117 breast tumors, 8 normal tissues	Over 14,000 genes
Study 7: Molecular Subtypes of Breast Cancer Are Associated with Characteristic DNA Methylation	GSE32392	Illumina GoldenGate Methylation Cancer Panel I	189 tumors, 4 normal breast tissues	807 cancer-related genes

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Supplementary Table II. Primer sequences used in qPCR analysis.

Gene		Forward primer (5'-3')	Reverse primer (5'-3')	Cat No.
<i>FGFR1</i>	qPCR	GCACATCCAGTGGCTAAAGCAC	AGCACCTCCATCTCTTTGTCCGG	HP225491
	COBRA	CCTCACTTTCCTTACAAACCRAACT	AAYGTAAAGGAGATTAGGTTGTAGGAAG	N/A
<i>FGF2</i>	qPCR	AGCGGCTGTACTGCAAAAACGG	CCTTTGATAGACACAACCTCCTCTC	HP205762
	COBRA	CAACTTAAAAAACRACCTCTCCCCAA	CCACTTCAAAAACCCCAAAACRACCTA	N/A
<i>GAPDH</i>	qPCR	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA	HP205798

Supplementary Table III. Association of FGFR1 and FGF2 expression with clinical features of breast cancer.

Patient ID	Tumor type	Grade	ER/PR/HER2 status	FGFR1 IHC score	FGF2 IHC score	Interpretation
1	IDC	II	ER+/PR+/HER2+	2+	2+	Moderate FGFR1, Moderate FGF2
2	Invasive Mammary	III	ER+/PR-/HER2+	2+	3+	Moderate FGFR1, High FGF2
3	Micropapillary	III	ER+/PR-/HER2+	2+	0	Moderate FGFR1, Negative FGF2
4	Invasive Mammary	III	ER-/PR-/HER2-	2+	1+	Moderate FGFR1, Low FGF2
5	Invasive Mammary	II	ER+/PR+/HER2+	0	3+	Negative FGFR1, High FGF2
6	IDC	III	ER-/PR-/HER2+	1+	3+	Low FGFR1, High FGF2
7	Micropapillary	III	NR/NR/NR	2+	2+	Moderate FGFR1, Moderate FGF2
8	IDC	High	ER+/PR+/HER2+	2+	3+	Moderate FGFR1, High FGF2
9	IDC	III	ER+/PR+/HER2+	1+	0	Low FGFR1, Negative FGF2
10	Invasive Mammary	High	ER+/PR+/HER2-	0	2+	Negative FGFR1, Moderate FGF2
11	IDC	II	ER+/PR+/HER2-	3+	3+	High FGFR1, High FGF2
12	Micropapillary	High	ER-/PR-/HER2-	2+	1+	Moderate FGFR1, Low FGF2
13	Invasive Mammary	High	ER+/PR+/HER2-	0	3+	Negative FGFR1, High FGF2
14	Micropapillary	II	ER+/PR-/HER2+	2+	3+	Moderate FGFR1, High FGF2
15	IDC	III	NR/NR/NR	2+	0	Moderate FGFR1, Negative FGF2
16	Micropapillary	III	NR/NR/NR	0	3+	Negative FGFR1, High FGF2
17	Micropapillary	III	ER+/PR+/HER2-	2+	2+	Moderate FGFR1, Moderate FGF2
18	Invasive Mammary	High	ER-/PR-/HER2+	2+	2+	Moderate FGFR1, Moderate FGF2
19	Invasive Mammary	High	ER-/PR-/HER2+	3+	1+	High FGFR1, Low FGF2
20	IDC	High	ER+/PR+/HER2+	2+	2+	Moderate FGFR1, Moderate FGF2
21	Micropapillary	High	ER-/PR-/HER2-	3+	0	High FGFR1, Negative FGF2
22	IDC	II	ER+/PR-/HER2+	0	3+	Negative FGFR1, High FGF2
23	IDC	High	ER-/PR-/HER2-	0	3+	Negative FGFR1, High FGF2
24	Micropapillary	III	ER-/PR-/HER2-	3+	1+	High FGFR1, Low FGF2
25	Micropapillary	II	NR/NR/NR	1+	0	Low FGFR1, Negative FGF2
26	Invasive Mammary	III	ER+/PR-/HER2+	1+	1+	Low FGFR1, Low FGF2
27	Invasive Mammary	High	ER+/PR-/HER2+	0	3+	Negative FGFR1, High FGF2
28	Micropapillary	III	ER-/PR-/HER2+	2+	3+	Moderate FGFR1, High FGF2
29	IDC	II	ER+/PR+/HER2+	3+	3+	High FGFR1, High FGF2
30	IDC	High	ER+/PR+/HER2-	3+	3+	High FGFR1, High FGF2

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Patient ID	Tumor type	Grade	ER/PR/HER2 status	FGFR1 IHC score	FGF2 IHC score	Interpretation
31	Invasive Mammary	III	ER+/PR-/HER2+	0	0	Negative FGFR1, Negative FGF2
32	IDC	III	ER-/PR-/HER2-	3+	1+	High FGFR1, Low FGF2
33	Micropapillary	III	ER-/PR-/HER2-	3+	2+	High FGFR1, Moderate FGF2
34	Micropapillary	III	NR/NR/NR	3+	1+	High FGFR1, Low FGF2
35	Micropapillary	III	ER+/PR-/HER2+	1+	2+	Low FGFR1, Moderate FGF2
36	IDC	II	ER+/PR-/HER2+	0	1+	Negative FGFR1, Low FGF2
37	Invasive Mammary	II	ER-/PR-/HER2-	3+	2+	High FGFR1, Moderate FGF2
38	IDC	II	ER-/PR-/HER2-	2+	1+	Moderate FGFR1, Low FGF2
39	Micropapillary	High	ER-/PR-/HER2-	1+	2+	Low FGFR1, Moderate FGF2
40	Invasive Mammary	III	ER+/PR-/HER2+	0	3+	Negative FGFR1, High FGF2
41	Invasive Mammary	High	ER+/PR+/HER2+	0	0	Negative FGFR1, Negative FGF2
42	Invasive Mammary	II	NR/NR/NR	0	1+	Negative FGFR1, Low FGF2
43	IDC	III	ER+/PR+/HER2-	0	2+	Negative FGFR1, Moderate FGF2
44	IDC	III	ER-/PR-/HER2-	3+	0	High FGFR1, Negative FGF2
45	Invasive Mammary	High	ER+/PR+/HER2+	2+	2+	Moderate FGFR1, Moderate FGF2
46	Invasive Mammary	II	ER+/PR-/HER2+	3+	2+	High FGFR1, Moderate FGF2
47	Invasive Mammary	II	ER-/PR-/HER2-	1+	3+	Low FGFR1, High FGF2
48	Invasive Mammary	II	NR/NR/NR	3+	2+	High FGFR1, Moderate FGF2
49	IDC	High	ER+/PR-/HER2+	1+	2+	Low FGFR1, Moderate FGF2
50	Invasive Mammary	II	ER+/PR-/HER2+	3+	2+	High FGFR1, Moderate FGF2
51	IDC	II	NR/NR/NR	1+	2+	Low FGFR1, Moderate FGF2
52	IDC	III	ER+/PR+/HER2-	0	2+	Negative FGFR1, Moderate FGF2
53	IDC	III	ER+/PR+/HER2-	3+	2+	High FGFR1, Moderate FGF2
54	IDC	II	ER-/PR-/HER2-	0	0	Negative FGFR1, Negative FGF2
55	Invasive Mammary	II	ER+/PR+/HER2-	2+	1+	Moderate FGFR1, Low FGF2
56	Invasive Mammary	II	NR/NR/NR	3+	2+	High FGFR1, Moderate FGF2
57	IDC	II	ER+/PR+/HER2+	1+	1+	Low FGFR1, Low FGF2
58	Micropapillary	III	ER+/PR+/HER2+	1+	0	Low FGFR1, Negative FGF2
59	Micropapillary	III	ER+/PR-/HER2+	1+	2+	Low FGFR1, Moderate FGF2
60	Micropapillary	III	NR/NR/NR	3+	3+	High FGFR1, High FGF2