Epigenetic regulation of ZEB1-RAB25/ESRP1 axis plays a critical role in phenylbutyrate treatment-resistant breast cancer

Supplementary Material

TableS1. PCR sequence of primers and fluorescent probe

method	Gene	Forward primer(5`>3`)	Fluorescent(5`>3`)	Reverse primer(5`>3`)
RT-PCR	β actin	TCACCCACACTGTG CCCATCTACGA		CAGCGGAACCGCT CATTGCCAATGG
RT-PCR	MUC L1	CTGGTACTCTTGGG AGTTTC		TTGGGTAAAACTGG AATGTCT
RT-PCR	RAB 25	ATGTTCGCTGAAAA CAATGG		GGGTGGACAGATAA AAGAGG
RT-PCR	TFA P2β	GTCCAGCCTATTGT TTGAGA		GTGCCGGTCCTCAT AGATA
RT-PCR	ESR P1	AATCGAAATGGCTT ATCCCC		GGTTGCATACTGGT AACCTT
RT-PCR /Q-MSP	ANK RD1	AGACAGAGAAGGA GATACCC	AGACTGAACCGCTATA AGATGATCCGA	CACCTATAACCCTG TGCTTT
RT-PCR	AXL	CACTTACAAGACTT GGTCCC		GGGAGCACTGTGAT GGT
RT-PCR	CAV 1	GTTCGAAGAGGTG GAGTG		TGTAGAGATGTCCC TGCG
RT-PCR /Q-MSP	ETS1	TTTTGGGAAGAAAG TCGGAT	AGCTCCAGATCGACTT TTTCCGTCTTG	CTGCACATTCCATA TCCGGG
RT-PCR /Q-MSP	IFI16	TCCTTACTGAGCAA CGATTT	CTCTGCTCCTTCAGTT TTGACAGTGC	CTGTTTTCGGGTTC TGAGC
RT-PCR /Q-MSP	KIAA 1199	GCATTTTCTTGGTA GCACAA	ATATATCTTTGTTCATG GTGATGCCTACAA	CGAGCAGTGACTTC TTGA
RT-PCR	PTR F	CATGATCTACCAGG ATGAAGT	CTTCTCCAGGTTCTCG CGGGTAC	TTTTCCTTGGTCTT GAGGC
Q-MSP	RAB 25	TGGGGAATGGAAC TGAGGAAGA	TCGCCGATCAGCACCA CCTTGAAGACA	CGTGAATCGGGAG AGTAGATTGG
Q-MSP	ESR	GGAGTTCGCCACA	TTTGAATCACCAGGGC	CAGACTTCATCTGG

RT-PCR /Q-MSP	P1	GATATTCGTA	CGCCCATCAG	ATAAAGGCATC
	ZEB1	AACAGTTGGTTTGG TGT	ATCAAGCCAATATTGC ATCC	CTTCACCCATACAA CAAGGT

*1 : RT-PCR was done at 95° C for 3 min followed by 30 cycles at 95° Cfor 1 min, 60° C for 1 min, 72° C for 1 min, and final extension at 72° C for 10 min.

1 μl dNTP mixture, 1.5 μl MgCl2 , 0.2 μmol/l each primer and 0.2 μl Platinum Taq DNA polymerase.

*2 : Bisulfite Sequencing PCR was done based protocol of EZ DNA Methylation-Gold Kit (QIAGEN).

*3 : Q-MSP was done at 95° C for 3 min followed by 40 cycles at 95° C for 20 sec, 60° C for 30 sec, and 72° C for 30 sec, in a 25 μl reaction volume containing 200nmol/l fluorescect probe, and 25 μl iQTM supermix.

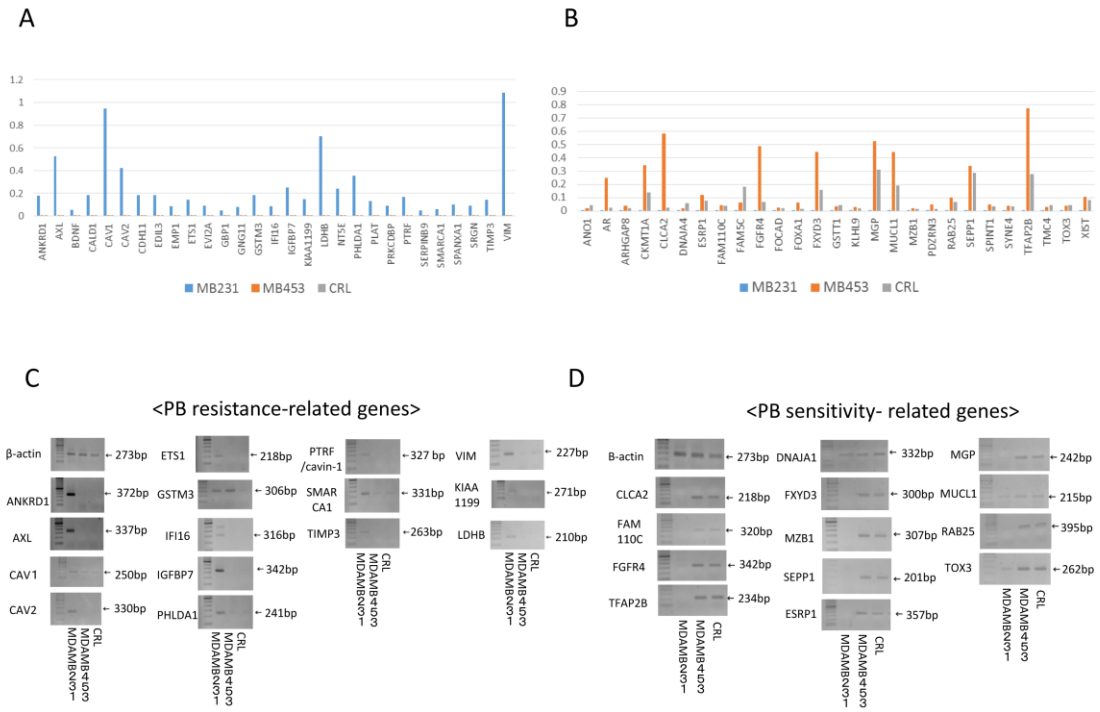

Figure S1. PB resistance-related genes and sensitivity-related genes identified using microarrays.

(A) PB resistance-related genes (those highly expressed in PB-resistant strains and minimally expressed in PB-sensitive strains) identified using microarrays.

(B) PB sensitivity-related genes (those highly expressed in PB-sensitive strains and minimally expressed in PB-resistant strains) identified using microarrays.

(C) mRNA expression levels of PB resistance-related genes in MDAMB453, CRL, and MDAMB231 cells as measured by semi-quantitative RT-PCR.

(D) mRNA expression levels of PB sensitivity-related genes in MDAMB453, CRL, and MDAMB231 cells as measured by semi-quantitative RT-PCR.