

Supplementary Material

Activation of TRAIL receptor genes expression following DNA demethylation in placental choriocarcinoma and transformed cell lines

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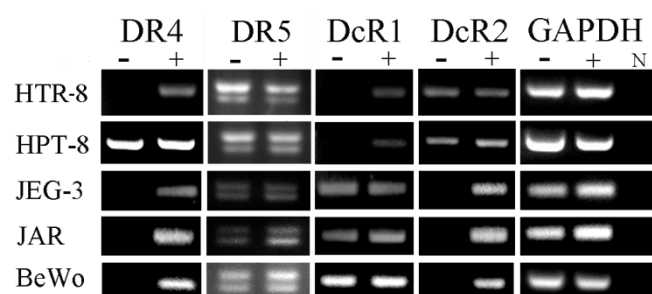


Fig. S1. RT-PCR analysis of restoration of the TRAIL receptors in cell lines with or without 5-aza-CdR.

cDNA was obtained from five human trophoblastic cell lines (JAR, JEG-3, BeWo, HTR-8/SVneo and HPT-8) in complete media with or without 5 μ M 5-aza-CdR (only HTR-8/SVneo was treated by 10 μ M 5-aza-CdR). (-) lanes indicate vehicle control; (+) lanes contain 5-aza-CdR. N, negative control reaction without template (using water instead of cDNA).

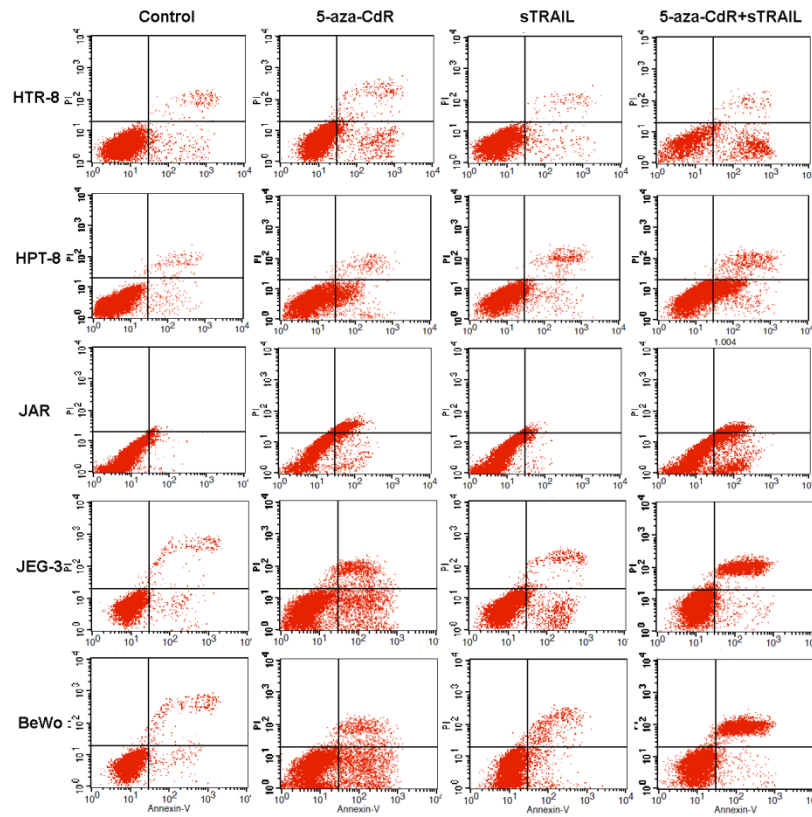


Fig. S2. Untreated trophoblast cells, or cells treated with 5-aza-CdR, TRAIL or both of 5-aza-CdR and TRAIL were stained with Annexin V and PI, and analysed by flow cytometry. Using the dual parametric dot plots combining Annexin V-FITC and PI fluorescence staining, the viable cell population was shown in the bottom left quadrant (Annexin V- PI-), and the early and late apoptotic cells were shown in the bottom right quadrant (Annexin V+ PI-), and the top right quadrant (Annexin V+ PI+), respectively.

Table S1. DR4 promoter methylation and gene expression

Cell line	Methylation level (%)		mRNA expression level (fold changes)	
	control	5-aza-CdR treated	control	5-aza-CdR treated
HTR-8	83.8	17.6	1	68.2±0.2
JAR	84.6	15.2	1	47.6±1.8
JEG-3	88.5	23.1	1	53.2±0.7
BeWo	80.0	17.6	1	60.3±0.9

Table S2. DcR1 promoter methylation and gene expression

Cell line	Methylation level (%)		mRNA expression level (fold changes)	
	control	5-aza-CdR treated	control	5-aza-CdR treated
HTR-8	92.4	25.7	1	63.5±0.3
HPT-8	93.3	28.0	1	41.3±0.2

Table S3. DcR2 promoter methylation and gene expression

Cell line	Methylation level (%)		mRNA expression level (fold changes)	
	control	5-aza-CdR treated	control	5-aza-CdR treated
JAR	92.7	19.6	1	40.1±0.1
JEG-3	89.3	17.8	1	68.4±1.1
BeWo	84.7	17.0	1	27.2±0.1