

Supplementary Material

Peperomin E Induces Promoter Hypomethylation of Metastatic-Suppressor Genes and Attenuates Metastasis in Poorly Differentiated Gastric Cancer

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Supplementary Materials

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Table S3. Primer list for methylation-specific PCR (MSP)

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Figure Captions

Fig. S1. ^1H -NMR (a) and ^{13}C -NMR (b) spectrum of Peperomin (PepE)

Fig. S2. HPLC peak purity analysis of PepE

Table S1. Primer list for RT-qPCR

Gene Symbol	Sense 5'-3'	Antisense 5'-3'	Size (bp)	Annealing temperature (°C)
h-DNMT1	CTACTCAGCCACCAAGAACGG	CAGGTTGCTGCCTTTGATGTAG	187	60
h-DNMT3a	GACAAGAATGCCACCAAAGCA	CCACTGAGAATTTGCCGTCTC	205	60
h-DNMT3b	GTGACACGGGGCTTGAATATG	CTTTCCTACCTTTATGCCCAACTC	116	60
h-E-cadherin	TGAAGGTGACAGAGCCTCTGGAT	TGGGTGAATTCGGGCTTGTT	200	60
h-TIMP3	CTGTGCAACTTCGTGGAGAG	GGCAGGTAGTAGCAGGACTTG	282	60
h-GAPDH	CCTCTGACTTCAACAGCGACAC	CTGTTGCTGTAGCCAAATTCGT	121	60

Table S2. PCR amplification protocol in RT-qPCR test

Steps	Cycle(s)	Temperature	Time	Detection
Initial denaturation	1	95°C	10 min	Off
Denaturation	40	95°C	5 sec	Off
Annealing and Extension		60°C	30 min	On

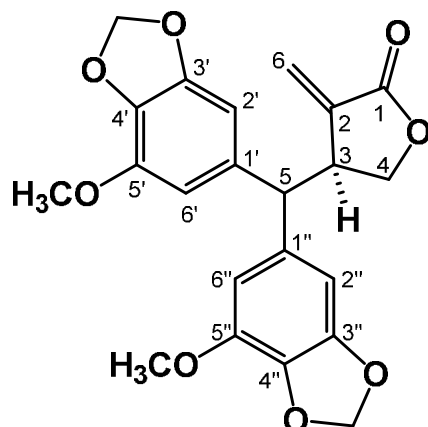
Table S3. Primer list for methylation-specific PCR (MSP)

Gene Symbol	Sense 5'-3'	Antisense 5'-3'
E-cadherin (M) ^a	TTAGGTTAGAGGGTTATCGCGT	TAACTAAAAATTCACCTACCGACC
E-cadherin (U) ^a	TAATTTTAGGTTAGAGGGTTATTGT	CACAACCAATCAACAACACA
TIMP3 (M) ^b	CGTTTCGTTATTTTTTGTTCGTTTTTC	CCGAAAACCCCGCCTCG
TIMP3 (U) ^b	TTTTGTTTTGTTATTTTTTGTTCGTTTT	CCCAAAAACCCACCTCA

- ^a. Guan ZY, Zhang J, Song SH, Dai DQ. Promoter methylation and expression of TIMP3 gene in gastric cancer. *Diagn Pathol.* 2013; 8: 110-6.
- ^b. Nam JS, Ino Y, Kanai Y, Sakamoto M, Hirohashi S. 5-Aza-2'-deoxycytidine restores the E-cadherin system in E-cadherin-silenced cancer cells and reduces cancer metastasis. *Clin Exp Met.* 2004; 21(1): 49-56.

Table S4. PCR amplification protocol in MSP test

Steps	Temperature	Cycle(s)	Time
Initial denaturation	95°C	1	10 min
Denaturation	95°C	40 cycles	30 sec.
Annealing	60°C		30 to 40 sec.
Extension	72°C		30 sec.
Final extension	72°C	1	7 min
Hold.	4°C		> 4 min



Peperomin E: ESI-MS m/z 435.1041 $[M+Na]^+$; **1H -NMR** δ_H 6.456, 6.443 (s, H-6', 6'', 2H), 6.379, 6.369 (s, H-2', 2'', 2H), 6.151 (d, H-6a, $J=1.8$ Hz, 1H), 5.934 (s, 4H, -OCH₂O-), 4.943 (d, H-6b, $J=1.5$ Hz, 1H), 4.321 (dd, H-4a, $J=7.5, 9.3$ Hz, 1H), 3.940 (dd, H-4b, $J=4.2, 9.3$ Hz, 1H), 3.887 (3H, s, -OCH₃), 3.897 (3H, s, -OCH₃), 3.74 (m, H-3, 1H). **^{13}C -NMR** δ_C 170.4 (C-1); 135.9 (C-2); 124.7 (C-6); 134.4, 134.3 (C-1, 1''); 149.4, 149.6 (C-3', 3''); 143.7, 143.5 (C-5', 5''); 136.1, 136.2 (C-4', 4''); 108.2, 108.4 (C-6', 6''); 101.7, 101.2 (C-2', 2''); 101.5 (-OCH₂O-); 42.5 (C-3); 55.3 (C-5); 56.9, 57.0 (-OCH₃); 69.7 (C-4).

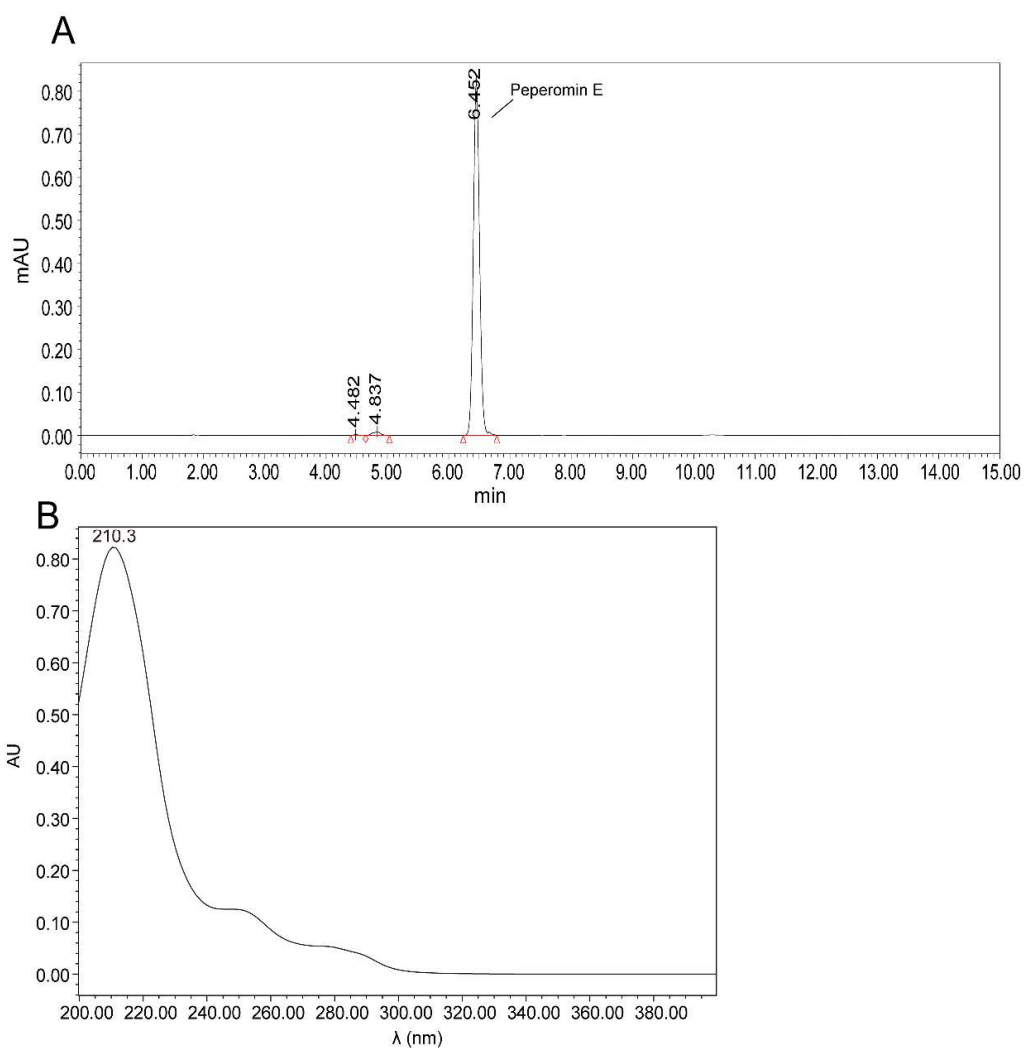


Fig. S2. HPLC peak purity analysis of PepE. (A) HPLC chromatogram of PepE, [the PepE was separated on a Water 2695 HPLC system coupled with a 2998 diode array detector (Waters, MA, USA), the chromatographic column used was Kromasil C₁₈ ODS column (250x4.6mm, 5 μ m) with column temperature at 30°C, the mobile phase was composed of acetonitrile: 0.1% phosphoric acid solution (40:60), the flow rate was kept at 1.0ml/min, UV absorbance range was monitored at 200~400 nm, peak purity threshold/peak purity angle=0.273/0.105=2.6]; (B) UV spectrum of PepE.