Supplementary Figure legends

Supplementary Figure S1

***A*,** western blot was prepared with 10 μg of protein extract from HTBOA cell line expressing *CTGF*, RMUG-S cell line lacking expression of CTGF, and either pCMV-3Tag4-CTGF- or empty vector- (mock) transfected HEK293T cells and anti-CTGF antibody. Specific signals for CTGF were detected in cells expressing endogenous CTGF protein or C-terminally 3xMyc-tagged CTGF protein.

B, representative results of an absorption test using synthetic peptide. Anti-CTGF antibody (2 μg/ml) was incubated with 0 or 20 μg/ml of synthetic peptide used as an antigen (sc-14939P, SantaCruz Biotechnology) overnight at 4°C, then used for immunohistochemstry using HTBOA and RMUG-S cell lines as positive and negative controls for *CTGF* expression, respectively. Note that positive staining was observed only in HTBOA cells without absorption by synthetic peptide.

Supplementary Figure S2

A, results of RT-PCR to reveal restored *CTGF* expression after treatment with 5-aza-dCyd for 5 days in OC cell lines (HTOA, HUOA, KF28, KFr13, MCAS, RMUG-L, RMG-II, and HMOA), which showed reduced expression of *CTGF* mRNA (Fig. 1C) and methylated pattern in Region 2 within the *CTGF* CpG island (Fig. 2B), except RMG-I and HNOA shown in Fig. 1 (***Upper***). No restoration of *CTGF* expression after treatment with 5-aza-dCyd was observed in RMUG-S with homozygous loss of this gene (***Lower***).

B, restored *CTGF* mRNA expression was observed in OVMANA and OVTOKO cells, which showed only unmethylated allele and unmethylated allele with partially methylated alleles in Region 2 within the *CTGF* CpG island by COBRA (Fig. 2B), respectively, after treatment with 5-aza-dCyd (5 or 10 μM) for 5 days or/and TSA (100 ng/ml) for 12 h (***Upper***).

Heterogenous and partial methylation pattern in Region 2 within the *CTGF* CpG island in OVTOKO cells was confirmed by bisulfite sequencing (***Lower***). See legend for Figure 2A for interpretation. Those results suggest that mechanisms other than methylation in promoter region, such as histone modification and epigenetic silencing of upstream components activating *CTGF* expression, may also contribute to the silencing of *CTGF*.

Supplementary Figure S3

Specificity and sensitivity of MSP for CTGF.

Upper, for MSP analysis, sodium bisulfite-treated CpGenome^{TM} Universal Methylated DNA (Chemicon International, sample *a*), DNA isolated from OSE-2a cells (sample *f*), or a mixture of those DNA with various combinations (sample *b*-*e*) was subjected to PCR using primers specific to the methylated (M) and unmethylated (U) forms of *CTGF* (Supplementary Table S1 and Fig. 2D). Almost no methylation within Region 2 of the *CTGF* CpG island in OSE-2a cells was confirmed by extensive bisulfite sequencing.

Lower, the same sample set of DNAs (*sample a*-*f*) was analyzed using COBRA. Although no methylated allele of *CTGF* was detected in both *sample e* and *f* by COBRA, MSP differentially detected the methylation of *CTGF* at a 0.1% level (*sample e*, *white arrow head*).