SUPPLEMENTARY INFORMATION

"Small Interfering RNA (siRNA)-Directed Reversal of uPA Demethylation Inhibits Prostate Tumor Growth and Metastasis"

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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. The combination of uPA siRNA did not induce DNA methylation within the *14-3-3σ* promoter in PC3 cells. PC3 cells were transfected with either control siRNA (siMM) or siRNA directed against the *uPA* gene promoter (siuPA). The methylation status of the CpG sites within the *14-3-3σ* promoter was determined by bisulfite sequencing.

Supplemental Figure S2. Effect of single siRNA targeting the *uPA* gene promoter on uPA mRNA (*top*) and protein expression (*bottom*). PC3 cells were transfected with mock, siMM or uPA siRNA. mRNA and protein expression of uPA and GAPDH were monitored by RT-PCR and immunoblot analysis, respectively. The number under each band is expressed as a percentage of Lipofectamine control (mock), normalized by the corresponding GAPDH level. MM, mismatched oligonucleotides.

Supplemental Figure S3. siRNA targeting the *uPA* promoter induced transcriptional silencing in PC3 prostate cancer cells. (A). Nuclear run-on assays of uPA transcription were carried out using nuclei from siRNA-transfected and mock cells as described in Methods. Specific hybridization to uPA was quantified using a PhosphorImager and was normalized to that of GAPDH. **(B)** Expression levels of *uPA* mRNA was determined by real-time RT-PCR. PC3 cells were transfected with mock, siMM or siuPA in the presence or absence of 5-aza and TSA. Results shown are the means ± SD of three independent experiments ($p<0.01$).

Supplemental Figure S4. siRNA targeting the *uPA* promoter induced DNA methylation in DU145 prostate cancer cells. (A) Bisulfite-based DNA methylation analysis using quantitative real-time PCR in DU145 cells. The PCR product amplified with methylation-insensitive primers was used for normalization. **(B)** Bisulfite sequencing analysis of the CpG island in the *uPA* promoter in mock-transfected cells and in cells transfected with siuPA or siMM.

Supplemental Figure S5. uPA silencing suppressed invasion and angiogenesis in DU145 prostate cancer cells. (A) The invasive capacity of cells transfected with mock, siMM or siuPA were assessed *in vitro* by matrigel invasion assay. **(B)** The representative number of invading cells through the matrigel was counted under the microscope in five random fields ($p<0.01$). **(C)** Angiogenic activity of cells transfected with mock, siMM or siuPA were determined by EC tube-like formation assay. Data are mean ± SD three independent experiments ($p<0.01$).

Supplementary Table 1

Sequence Name	**Sequence (5'-3')**
uPA siRNA–1	GCA CGG AGA ATT TAC AAG CCT
uPA siRNA–2	TCT TTG TGA GCG TTG CGG AAG
uPA siRNA–3	GCA CGC GGG GTC CGG GTC GCT
uPA siRNA–4	AGG GGC GGC GCC GGG GCG GGC
uPA siRNA–1 with mismatches	GGA CGG ACA ATT TAG AAG GCT
uPA siRNA–2 with mismatches	TGT TTG TCA GGG TTG GGG AAG
uPA siRNA–3 with mismatches	GCA GGC GGC GTG CGG GTG GCT
uPA siRNA–4 with mismatches	AGG GGG GGG GCG GGG GGG GGC

Mismatched bases in uPA siRNA1-4 are underlined.