Epigenetic Upregulation of Urokinase Plasminogen Activator Promotes the Tropism of Mesenchymal Stem Cells for Tumor Cells

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. uPA expression levels in CB- and BM-MSCs.

(A) RNA samples extracted from MSCs isolated from two CB and two BM cases were analyzed using RT-PCR for uPA expression levels. GAPDH mRNA was amplified as a loading control and expression standard.

(B) Methylation-specific PCR (MSP) analysis of the *uPA* gene promoter region in CB- and BM-MSCs. Bisulfite-modified DNA derived from MSCs were amplified with uPA primers specific for unmethylated and methylated DNA. uPA-U: unmethylated PCR product; uPA-M: methylated PCR product. Positive controls used for MSP included DNA from PC3 cells as unmethylated DNA control and CpGenome Universal methylated DNA as methylated DNA control (Chemicon Internaltional). Negative control MSP reactions were performed using water only (no DNA) as template

Supplementary Figure S2. Effect of trichostatin A (TSA) on the accumulation of acetylated histone H4 in CB- and BM-MSCs. Immunofluorescence analysis of the acetylation status of

histone H4 in MSCs from representative CB and BM samples treated with 100 nM TSA for 16 hrs, immunostained with anti-acetylated histone H4 antibody (FITC, green), counterstained with Hoechst to visualize nuclei (blue), and analyzed at 40x magnification. Untreated cells served as controls.

Supplementary Figure S3. Effect trichostatin A (TSA) on cellular migration of BM-MSCs.

(A) The migration capacity of the control and TSA-treated BM-MSCs toward human cancer cells PC3 and MDA-231 were assessed *in vitro* by transwell migration assay. An uPA antibody caused a significant decrease in TSA-treated BM-MSCs migration to tumor cells. Columns, percentage of the DMEM control (*p <0.05); bars, SD.

(B) The migration capacity of the control and TSA-treated BM-MSCs toward non-tumor cells RWPE1 and HEK293 was assessed as described in (A).

Supplementary Figure S4. uPA expression induces phospho-ERK levels in CB-MSCs.

(A) Immunoblot analysis shows increased levels of ERK1/2 phosphorylation in uPA-overexpressing MSCs (uPA-2). GAPDH was used as a loading control.

(B) ERK expression levels in control (CTL) and TSA-treated CB-MSCs were analyzed by immunoblot. GAPDH was used as a loading control.