Supplementary Materials and Methods, Figure Legends

Materials and Methods

Proliferation assay

Cell proliferation was measured using the Cell-Titer 96® AQueous One Solution Cell Proliferation Assay (MTS) kit (Promega, Madison, WI, USA). U937 cells (2×10^4) and HL60 cells (5×10^4) in 100 µl RPMI 1640 medium containing 10% FCS were seeded into 96-well plates in triplicate. MTS reagent (20 µl) was added to each well at 0, 24, 48, 72 hours and, after one to two hours incubation, the absorbance at 490nm was measured. Cell proliferation of individual samples was calculated by normalizing their absorbance to that of the corresponding control sample.

Colony forming assays

Transfected U937 and HL60 cells (5×10^3 cells/ml each) were seeded into 0.9% methylcellulose medium. The number of colonies containing more than 50 cells in each well was counted on day 14.

Animal xenograft tumor model

A suspension of 3×10^7 transfected U937 cells in 200 µl PBS was injected subcutaneously into the right flanks of nu/nu athymic nude mice (n = 7). Tumor volumes were measured every 3 days in two dimensions using vernier calipers. Tumor volumes were calculated using the formula: (length × width^2) × 0.5. After 21

days, mice were sacrificed by cervical dislocation under anesthesia. All animal experimentation was approved by the institute's Animal Research Committee.

Apoptosis analysis

Transfected U937 and HL60 cells were stained with PE-labeled Annexin-V and 7-AAD (BD Pharmingen) according to the manufacturer's instructions. The FACS assay was performed immediately after staining.

Figure legends

Figure S1. Induction of mda-7/IL-24, IL-24 delE5 and differentiation markers in U937 and HL60 cells treated with TPA. U937 and HL60 cells were treated with 20 nM TPA for 0, 24, 48, or 72 hours. (A) Real-time PCR was performed using specific primers for mda-7/IL-24, IL-24 delE5 and GAPDH. Results were expressed as the mean ± S.D. of three independent experiments ($p < 0.05$). (B) Monocytic surface markers CD11b, CD14 and CD115 were analyzed by FACS. Results were expressed as the mean ± S.D. of three independent experiments ($p < 0.05$).

Figure S2. RNA interference of the mda-7/IL-24 and IL-24 delE5 gene blocks TPA-induced monocytic differentiation of U937 and HL60 cells. U937 and HL60 cells were transfected with 100 nM siRNA corresponding to mda-7/IL-24, IL-24 delE5, or non-targeting siRNA (NT). After transfection, differentiation was induced by addition of

TPA. After 72 hours, cellular morphological changes associated with differentiation were detected with Wright-Giemsa staining. Representative micrographs from three independent experiments with similar results are shown (Leica DM4000B microscope, 40×/0.75 HCL PL objective lens, Leica DC500 digital camera. Original magnifications ×1 000).

Figure S3. p38 MAPK and JNK pathway are not involved in regulation of TPA-induced mda-7/IL-24 and IL-24 delE5 expression in U937 and HL60 cells. (A) U937 and HL60 cells were treated with TPA for 12 hours. The phosphorylated, total p38 and JNK proteins were determined by western blotting using specific antibodies. (B) U937 and HL60 cells were pretreated with or without the JNK inhibitor SP600125 (10 μM) for 30 minutes and subsequently exposed to TPA for 48 hours. The relative mRNA expression levels of mda-7/IL-24 and IL-24 delE5 were analyzed by quantitative real-time RT-PCR. Results were expressed as the mean ± S.D. of three independent experiments from each cell line ($p > 0.05$).

Figure S4. Ectopic overexpression of mda-7/IL-24 and IL-24 delE5 exerts significant antileukemic effect in vitro and in vivo. U937 and HL60 cells were transfected with mda-7/IL-24, IL-24 delE5, or the empty vector as a negative control. (A) Cell viability was determined using MTS at the indicated times. The experiments were performed in triplicate and repeated three times, and results were expressed as the mean ± S.E ($p < 0.001$). (B) U937 cells were subcutaneously injected into the right flank of seven

nu/nu mice. Tumors were measured at the indicated times, and calculated volumes (mean ± S.D.) are presented as growth curves ($p < 0.001$). (C) The number of colonies was counted. Results were expressed as the mean ± S.E., and all experiments were performed twice using triplicate plates for each experimental point ($p < 0.001$).

Figure S5. Ectopic overexpression of mda-7/IL-24 and IL-24 delE5 has no effect on apoptosis of U937 and HL60 cells. U937 and HL60 cells were transfected with mda-7/IL-24 and IL-24 delE5, the empty vector as a negative control. Cells were harvested 72 hours after transfection, and stained with PE-labeled Annexin-V and 7-AAD and immediately analyzed by FACS. Representative results from three independent experiments with similar results are shown ($p > 0.05$).