Supporting Information

Mass spectrometric quantification of microRNA in biological samples based on multistage signal amplification

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Experimental

MCF-7 Cell Culture.

The MCF-7 cells were harvested from culture flasks during mid-logarithmic growth with trypsin/EDTA, washed, resuspended in DMEM and counted. Then transfer to thermo scientific™ nunc™ cell-culture treated multi-dishes. 50 μL cell suspension was delivered to each 1.0-mm well of multiple plates. Cell-culture treated multi-dishes were prepared to provide replicate sample wells for the control and drugs at each time point studied. The multi-dishes were kept in an incubator at 37°C in a water-vapor-saturated atmosphere containing 5% CO_2. The cells were seeded and allowed to grow for 3-4 days to achieve logarithmic growth before addition of the drug tested. The medium was then replaced daily to ensure that nutrient depletion would not affect cell growth.

Drug Treatment.

Toremifene 2-{p-[(Z)-4-chloro-1,2diphenyl-1-butenyl] phenoxy}-N, N-dimethylethylamine citrate (1:1) was dissolved in 70% ethanol and stored as "stock" solutions at -20°C until used. The final concentration of ethanol in drug-treated cultures was always less than 0.1%. The control cultures were fed with DMEM containing the same ethanol concentration as the drug-treated wells. To study changes of intro miR-21 level caused by drug stimulus, MCF-7 cells prepared as above were incubated with toremifene from 1μM to 10 μM in culture media for 6 h. The images of cells exposed to stimulus are shown in Figure S2. The cells were collected to measure miR-21. In order to decrease random error, cells collected from 4 wells via three steps of HE, CEA and AH, and then using HPLC-MS/MS to analysis.

Supplementary Table and Figures

Table S1

Table S1 Oligonucleotide sequences used in this work.

Name	Sequence (5'- 3')
miRNA-21	UAG CUU AUC AGA CUG AUG UUGA
SBM-21	UAG CUU AUC AGA CUG AU*A* UUGA
TBM-21	UAG CUU AUC AGA CUG *CGG* UUGA
Signal probe	Biotin -C3-TCAA CAT CAG TCT GAT AAG CTA CCCCCC
Test probe	TCAA CAT CAG TCT GAT AAG CTA CCCCCC

Figure S1

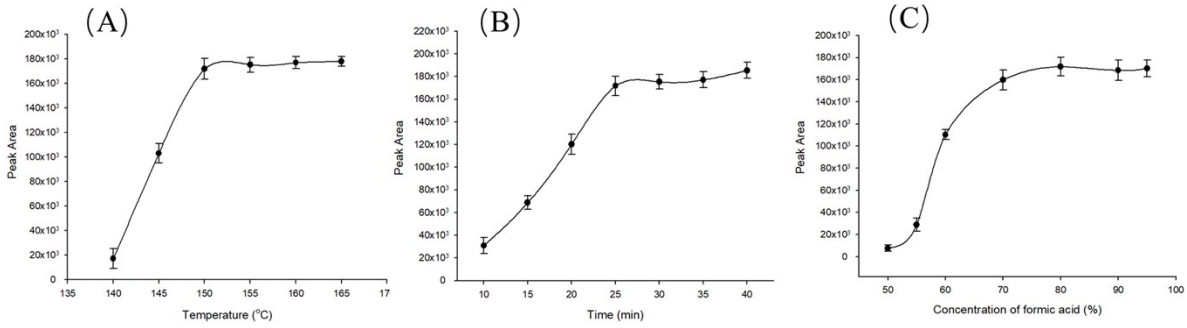

Fig. S1 Study of experimental conditions for acid hydrolysis: effects of temperature (A), hydrolysis time (B), and formic acid concentration (C).

Figure S2

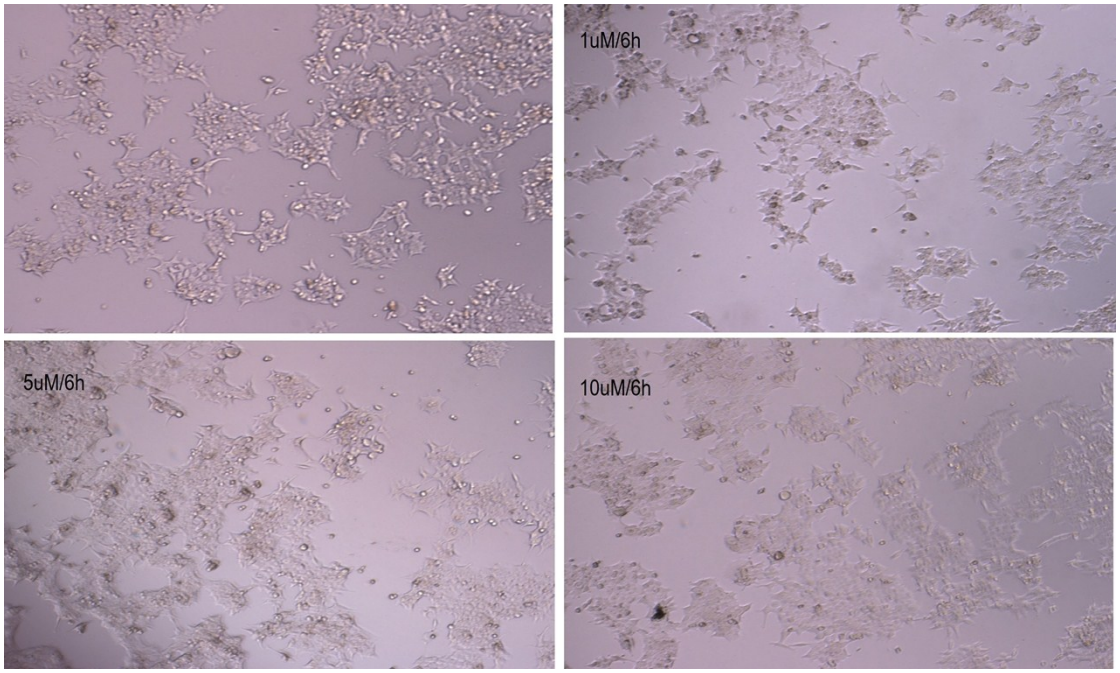

Figure S2. Microscopic images of MCF-7 cells after being cultured for 6 h in media. Concentrations of toremifene were 0, 1.0, 5.0, and 10.0 μM, respectively.