Additional_file_1

Table S1. List of primers used in the study

Gene name	Seqence (5' - 3')	
	Fwd	Rev
ECM1	CCC ACC CAT CTG AAC ACT CAT TAC	CAG GGC ACC AAG ATC CAC TTA AA
EGFR	ATA GTC GCC CAA AGT TCC GTG AGT	ACC ACG TCG TCC ATG TCT TCT TCA
HER2	GCT GAA CAA TAC CAC CCC TGT CAC AG	TGT GAG AGC CAG CTG GTT CTT G
HER3	AAT GGC TCG GGC TCT GAT ACT TGT	TAC TTG TAG ATT GGG CCC TTG GCA
MUC1	CTG CTC CTC ACA GTG CTT ACA GTT G	TGA ACC GGG GCT GTG GCT GG
MMP-9	ACT GCT GGC CCT TCT ACG GC	CTC CCC CTG CCC TCA GAG AA
GAPDH	TCG ACA GTC AGC CGC ATC TTC TTT	ACC AAA TCC GTT GAC TCC GAC CTT

Supplementary materials and methods

Three-dimensional growth assays

Growth factor-reduced matrigel (BD Biosciences), diluted 1:3 with DMEM and containing 5 x 10^4 cells/ml, was poured into 6-well plates, which were then incubated at 37ºC in 5% CO2 and 95% humidified air. The medium was replaced with DMEM containing trastuzumab every 3 days and colonies were photographed after 12 days. Cell colonies measuring over 20 μm were counted using a biological microscope equipped with OLYMPUS cellSens Standard 1.5 software.

Soft agar colony forming assays

Colony forming assay using low-melting point agarose were done as following description in the presence of trastuzumab. Bottom layers containing DMEM with 10% FBS were consist of 0.8% of low-melting point agarose (Sigma-Aldrich) and 10 mM HEPES. Each bottom layers were poured on 6-well plates and solidified for 30 min at room temperature. Fifty thousand cells were seeded in top layers containing DMEM with 10% FBS, 0.4% low-melting point agarose and 5 mM HEPES. Colonies were photographed after 12 days.

Reverse transcriptation-polymerase chain reaction (RT-PCR)

Expression of transcripts was assessed using the following primers, human ECM: Fwd 5'-AGG CTC GGT TCT CCT GCT TCC AG-3', Rev 5'-TTG GGG TAA GGA GCC CGA CGG-3' and human GAPDH primers: Fwd 5'-GGT GAA GGT CGG AGT CAA CG-3', Rev 5'-CAA AGT TGT CAT GGA TGA CC-3'.

Measure of miRNA expression

Total RNA was isolated from cells using the mirVana MicroRNA isolation kit (Lifetechnologies). cDNAs were prepared from 2 μg total RNA using the TaqMan MicroRNA Reverse transcription kit (Lifetechnologies). Expression of miR-200c was assessed by RT–qPCR with a universal reverse primer and forward primers specific for miR-200c using the TaqMan MicroRNA Assays kit (Life technologies).

Zymography

Zymography was performed on 10% polyacrylamide gels that containing 0.5 mg/mL gelatin (Sigma-Aldrich). Samples were resuspended in a zymography sample buffer without denaturation and run on a gel containing gelatin. After electrophoresis, the gels were incubated for 30 min at room temperature in a renaturing buffer (50 mM Tris; pH 7.4, 2% Triton X-100). After renaturation, the gels were incubated for 48 h at 37°C in developing buffer (50 mM Tris; pH 8.0, 5 mM $CaCl_2$, 0.02% NaN_3). After developing, the gels were stained with 0.1% Coomassie Blue R-250 for 30 min at room temperature and then the gels were destained with destaining solution (25% ethanol, 10% acetic acid).