Supporting Information

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SI Materials and Methods

Antibodies and Reagents. Antibodies specific for phospho-ERK (P-ERK) and P–Tyr-705 in STAT3 were from Cell Signaling Technologies. Antibodies that detect total ERK, P–Tyr-1173 in the EGF receptor (EGFR), STAT5b, and P-STAT5b were from Millipore. Urokinase-type plasminogen activator (uPA)-specific monoclonal antibody was from American Diagnostica. Human uPA receptor (uPAR)-specific antibody was from R&D Systems. Antibody that detects P–Tyr-845 in the EGFR and BrdU conjugated with Alexa Fluor 594 were from Invitrogen. BrdU was from BD Biosciences. Horseradish peroxidase-conjugated anti-rabbit IgG and anti-mouse IgG were from GE Healthcare. The Src family kinase (SFK) inhibitor 4-amino-5-(4-chlorophenyl)-7-(dimethylethyl)pyrazolo[3,4-*d*]pyrimidine (PP2) and the EGFR inhibitor AG1478 were obtained from EMD Biosciences. Quantitative PCR (qPCR) reagents, including primers and probes for uPA, uPAR, and hypoxanthine phosphoribosyltransferase 1 (HPRT-1), were from Applied Biosystems. Purified uPA was a gift from Andrew Mazar (Northwestern University, Evanston, IL). Human EGF was from R&D Systems. Erlotinib and gefitinib were from LC Laboratories.

Real-Time qPCR. Total RNA was isolated with the RNeasy Kit (Qiagen). cDNA was synthesized with the iScript cDNA Synthesis Kit (Bio-Rad). qPCR was performed with a System 7300 instrument (Applied Biosystems) and a one-step program: 95 °C for 10 min, 95 °C for 30 s, and 60 °C for 1 min for 40 cycles. HPRT-1 gene expression was measured as a normalizer. Results were analyzed by the relative quantity method. Experiments were performed in triplicate with internal duplicate determinations.

Immunoblot Analysis. Cell extracts were prepared in RIPA buffer [20 mM sodium phosphate, 150 mM NaCl (pH 7.4), 1% Nonidet P-40, 0.1% SDS, and 0.5% deoxycholic acid] containing complete protease inhibitor mixture (Roche). Protein concentrations were determined by bicinchoninic acid assay (Sigma-Aldrich). Equal amounts of cell extracts were subjected to SDS/PAGE, electrotransferred to PVDF membranes, and probed with primary antibodies.

siRNA Transfection. uPAR-specific siRNA (5′-GCCGUUACCUCGAAUGCAU-3′) and uPA-specific (5′-CAUGUUACUGACCAGCAAC-3′) siRNA have been previously described (1, 2). The nontargeting control (NTC) siRNA pool was purchased from Dharmacon. siRNAs (25 nM) were introduced into cells by incubation with Lipofectamine 2000 (Invitrogen) in serum-free medium (SFM) for 4 h. Cultures were allowed to recover in serum-containing medium for 12 h. The extent of gene silencing was determined by qPCR and immunoblot analysis.

Cell Growth. Cell growth was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells (1×10^5 cells per well) were plated in 12-well plates and cultured for 48 h in SFM. MTT was incubated with the cells for 4 h. MTT hydrolysis was determined by the absorbance at 570 nm. Cultures were analyzed before and after the 48-h incubation in SFM.

BrdU Incorporation. Cell proliferation was measured by BrdU incorporation (2). Cells were cultured on coverslips for 12 h, washed with SFM, and then cultured in SFM for 2 h before adding BrdU (100 μM) for 6 h. Cells were fixed with formalin and treated with 2 M HCl for 1 h at 37 °C. Fixed cells were incubated with Alexa Fluor 594-conjugated BrdU-specific antibody (1:50). Preparations were examined with a Leica DMIRE2 fluorescence microscope. The rate of cell proliferation was determined as the fraction of BrdU-positive cells. At least four fields in three different coverslips were reviewed. All experiments were performed in triplicate. Statistical significance was determined by unpaired *t* test.

Cell-Death Assay. Cell death was determined with the Cell Death Detection ELISA Kit (Roche Applied Science). Cells were cultured in SFM for 48 h before performing assays.

1. Jo M, et al. (2009) Reversibility of epithelial-mesenchymal transition (EMT) induced in breast cancer cells by activation of urokinase receptor-dependent cell signaling. *J Biol Chem* 284:22825–22833.

2. Jo M, et al. (2007) Urokinase receptor primes cells to proliferate in response to epidermal growth factor. *Oncogene* 26:2585–2594.