Supplementary Information

Supplementary Methods

Cell Culture

The immortalized human mammary epithelial cells (HMLE) and the transformed cells (HMLE-Ras) were kindly provided by Dr. Robert A. Weinberg, and maintained as described (1). MCF10A mammary epithelial cells were cultured as previously described (2). For PiB treatment, cells were exposed to 1 mM PiB for three days. Freshly sorted primary mouse mammary epithelial cells were cultured in DMEM/F12 medium supplemented with 20 ng/ml of EGF, 10 mg/ml of insulin, 0.5 mg/ml of hydrocortisone, 1% bovine serum albumin, and 2% calf serum (3). Freshly isolated primary normal human MEC or breast cancer cells were cultured in MEGM with supplements (4).

Generation of Stable Cell Lines

For overexpression, Pin1 CDS were subcloned into the pBabe retroviral vector or pBybe lentiviral vector. Specific point mutations were introduced using the Quickchange kit (Stratagene) and sequences were verified. All lentiviral shRNA constructs were provided by Dr. William C. Hahn. The production of retroviruses or lentiviruses as well as the infection of target cells was described previously (5). Following infection, the cells were selected using hygromycin or puromycin. Cells were used immediately following selection and for up to three weeks after selection. Fresh stable cell lines were made before each group of experiments and experiments were performed following at least two separate infections.

In Vitro Assays

Mammosphere culture was performed as described (6). Single-cell suspension was plated on ultra-low attachment plates (Corning, Costar) in DMEM/F-12 HAM medium containing bFGF, EGF, heparin and B-27 supplement. The mammospheres were cultured for two weeks. Then the mammospheres with diameter >75 μm were counted.

For wound healing assays, cells were grown to confluence and then wounded using a yellow pipette tip tip, and migration was visualized by time-lapse imaging. The rate of wound closure was calculated by a ratio of the average distance between the two wound edges and the total duration of migration.

Transwell migration assay were performed as previously described(7). Assay media with EGF (5 ng/ml) was added to the bottom chamber. Cells (5×10^4/100 μl) were added to the top chamber of cell culture inserts (8 mm pore size) (Corning, Costar). After 12 h of incubation, cells that migrated to the bottom surface of the insert were fixed with methanol and stained with 0.4% crystal violet. The number of cells that had migrated was quantified by counting ten random distinct fields using a microscope.

Western Blot

Primary monoclonal Pin1 antibody (1:5000), monoclonal M2 antibody for Flag tag (1:2000) (Sigma) and monoclonal Actin antibody (1:5000) (Sigma) are used.

Quantitative RT-PCR

RNA from sorted cells was extracted with the Total RNA isolation mini kit (Agilent). cDNA was prepared with transcriptor first strand cDNA synthesis kit (Roche) and PCR was carried out with iQ SYBR Green Supermix (Bio-Rad). Samples were run on the QIAGEN Rotor-Gene Q real-time cycler. GAPDH was used as an internal control. Analysis was performed with the delta-delta ct method. Primers for let-7 miRNA were from Ambion. The following primers were used:

GAPDH forward CATGAGAAGTATGACAACAGCCT

GAPDH reverse AGTCCTTCCACGATACCAAAGT

Pin1 forward GCCTCACAGTTCAGCGACT

Pin1 reverse ACTCAGTGCGGAGGATGATGT

Ecad forward TGCCCAGAAAATGAAAAAGG

Ecad reverse GTGTATGTGGCAATGCGTTC

Ncad forward ACAGTGGCCACCTACAAAGG

Ncad reverse CCGAGATGGGGTTGATAATG

FN1 forward CAGTGGGAGACCTCGAGAAG

FN1 reverse TCCCTCGGAACATCAGAAAC

Vim forward GAGAACTTTGCCGTTGAAGC

Vim reverse GCTTCCTGTAGGTGGCAATC

Preparation of Single-cell Suspensions

Mouse mammary glands, human breast tissues and breast cancer tissues were mechanically disaggregated and then digested with 200 U/ml collagenase (Sigma) and 100 U/ml hyaluronidase (Sigma), as described (8). The resultant organoids were further digested in 0.25% trypsin-EDTA and Dispase/DNaseI, and then filtered through a 40 μm mesh.

SUPPLEMENTAL REFERENCES

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