Patient–derived tumor grafts authentically reflect tumor pathology, growth, metastasis, and disease outcomes

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SUPPLEMENTARY METHODS

Tumor processing. Harvested tumors were divided into pieces and either flash frozen in liquid nitrogen, fixed in 4% paraformaldehyde and embedded in paraffin, or cryoprotected consecutively with 5%, 15% and 30% sucrose prior to embedding in Tissue-Tek OCT embedding medium (Sakura). In order to approximate the frequency of metastasis, we performed hematoxylin-eosin staining and immunohistochemistry on sections from entire organs (lungs and lymph nodes) upon necropsy, systematically staining 3 serial sections every 25 microns. Frequencies of metastasis were determined at the time of necropsy, which corresponds to an average tumor growth time of 4 months for HCI-009 and HCI-011 (to achieve 2 cm primary tumors), and 5 months for HCI-005 (to achieve 1 cm primary tumors) (see **Supplementary Table 1**). No primary tumor resection was required to achieve these frequencies of metastasis. The minimum latency required for metastases to be detectable has not been determined.

Histology and immunostaining. Staining was performed using standard microtome sectioning, deparaffinization and hydration, and antigen retrieval in either boiling 10mM sodium citrate buffer pH6.0, DAKO citrate buffer (Dako), or Tris-EDTA buffer (pH 9.0), depending on the primary antibody to be used. After blocking endogenous peroxidases with H_2O_2, endogenous biotin was saturated with a biotin blocking kit (Vector Laboratories). The sections were then treated with blocking solution (DAKO EnVision+System for rabbit antibodies, Vector MOM kit

for mouse antibodies, or 10% human serum, depending on the primary antibody to be used) and incubated with primary antibodies from 1 hour to overnight at room temperature, also depending on the antibody. Sections were washed in 0.5% Tween-PBS solution and incubated with secondary antibody (DAKO EnVision+System–HRP kit) for 30 min at room temperature. Staining was visualized by 3,3-diaminobenzidine, with hematoxylin as a counter-stain (Mayer's hematoxylin, Sigma-Aldrich). The following antibodies were used: anti-CD31 (1:50, Abcam #ab59251); anti-ER alpha (1:100, Epitomics #4200-1 or 1:500, Epitomics Clone #SP1); anti-caspase 3 (1:100, Cell Signaling #9661); anti-phospho-histone H3 (1:100, Cell Signaling #9701); anti-Ki67 (1:50, Santa Cruz #sc-15402); anti-cytokeratin (1:500, DAKO #Z0622); anti-β-catenin (rabbit polyclonal 1:200, Cell signaling #9562 or mouse monoclonal 1:50, Abcam clone #17c2); anti-vimentin (1:50, Developmental Studies Hybridoma Bank, University of Iowa); and anti-PR (Dako #A0098). Staining for ER, HER2 and E-cadherin for characterization of clinical specimens and xenografts was performed side-by-side by ARUP Labs (Salt Lake City, UT) using standard clinical assays.

Slides were read by a board-certified clinical breast pathologist (R.F.), who was blinded to the sample identities and sources (mouse or human). The presence of invasive carcinoma was assessed, and histology of the tumors was compared using the modified Scarff-Bloom-Richardson (MSBR) grading system for invasive breast carcinomas[2]. Features of the tumors not included in the MSBR system were also noted. Immunohistochemistry results were evaluated according to standard practice.

Lentivirus transduction. HIV-ZsGreen lentiviral particles were prepared as described[3] and added to the hMSC cultures (multiplicity of infection of 5) with polybrene (8ug/ml) and

incubated overnight. The medium containing lentiviral particles was then removed and fresh medium was added. The infected cells were sorted by FACS for GFP+ cells.

RNA and DNA isolation. For RNA extraction, about 50mg of flash frozen tumor tissue was homogenized in 1 ml TRIzol reagent (Invitrogen) at high speed until all tissue was completely broken down. The RNAs from the aqueous phase were precipitated with isoropanol (J.T.Baker) and the pellets were washed with 70% ethanol. After briefly air-drying, RNA pellets were dissolved in RNase-free water. Only RNAs with an RNA integrity number (RIN) above 7.5 and $A_{260/230}$ ratios from 1.9-2 were used for gene expression microarray experiments. The DNeasy Blood & Tissue Kit (Qiagen) was used for DNA isolation from about 25mg frozen tumor, according to the manufacturer's recommendations. To obtain RNA-free genomic DNA, RNase A (Qiagen) was added into the tissue mixture. Only isolated DNAs with $A_{260/280}$ and $A_{260/230}$ ratios above 1.8 and proven to be high quality by gel electrophoresis were used for SNP microarray analysis.

Protein isolation. Tumors were lysed by homogenizing in Buffer B (25mM Tris-HCl, pH 7.5, 0.42M NaCl, 1.5mM $MgCl_2$, 0.5mM EDTA, 1mM DTT, 25% sucrose, 1mM Na_3VO_4, and 1X protease inhibitor cocktail), followed by centrifugal clearing at 4^{o}C for 10min at 10,000 rpm to recover whole cell lysates.

Western blotting and ER quantification. Tumor protein extracts were serially diluted and run on a 10% SDS-PAGE gel, then transferred to PVDF membrane for standard Western blotting. ER was detected using anti-ERα (1:500, Epitomics #4200-1). Levels of ERα were quantified and normalized to β-actin using Image J software.

Gene expression microarray analysis and sample clustering. Total RNA samples were labeled with Cy3 fluorescent dye and hybridized to Agilent Human 44k and 24k whole−genome

expression arrays using standard Agilent methods. Arrays were scanned at 5 μm resolution using an Agilent Technologies G2505C Microarray Scanner. Images were analyzed using Agilent Feature Extraction software (version 10.5). Quality control reports for each array were examined, and all arrays passed Agilent's quality metrics. Microarray features flagged as non−uniform on population outliers were removed, and the remaining features were coalesced into microarray probe−specific intensity values by averaging. Data was transformed to the $\log_2$ scale. Log−scale intensity values were quantile−normalized using software from the Bioconductor software suite in R. Normalized log−scale intensity values were analyzed for differential expression in GeneSifter.

Unsupervised gene expression analysis of parental breast tumors and tumorgrafts. The gene expression data described above were merged with Agilent microarray data for samples presented in Parker et al[4]. We found 1,291 "intrinsic" genes in common between the Parker et al dataset and our dataset by matching either probe name or systematic name. Data from multiple probes on the array for the same gene were collapsed to a single value by calculating the mean. Each dataset was imputed using K−nearest neighbor (KNN) and then merged using distance weighted discrimination, as previously described in Hu et al[5]. Data was clustered using Pearson correlation, median centering by gene/array, and associating by centroid linkage[6]. Data for the pre−clustered "intrinsic" gene set after merging are available with the online version of the paper (**Supplementary Table 4**).

SNP array experiments and copy number variant analysis. Genomic DNA samples were labeled using the standard Affymetrix StyI labeling protocol, and were hybridized to Affymetrix SNP 6.0 arrays. Arrays were scanned on an Affymetrix 3000 7G Microarray scanner. The resulting images were processed using the Affymetrix GeneChip Operating Software version 1.4,

and results were saved as CEL files. The CEL files were imported into Partek Genomics Suite software for analysis using the Copy Number Workflow. For each sample (whether original tumor or subsequent tumorgraft(s)) the copy number of each microarray probe was calculated using a pool of five normal DNA samples as a reference.

REFERENCES FOR SUPPLEMENTARY METHODS

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