Supplementary Materials and Methods

siRNAs

siRNAs were obtained from Qiagen and reverse transfected into cells using RNAiMAX (Invitrogen) to a final concentration of 10nM. See Supplementary methods for sequences. with the following sequences: siBRCA1: 5'-CAG GAA ATG GCT GAA CTA GAA-3`; siBRCA1 #2: 5'- ACC ATA CAG CTT CAT AAA TAA -3'; siSCR: 5'-AGC AGC ACG ACT TCT TCA AGT-3'; siCYP1A1: 5' – CAGCAAGTTAGAACTAGCCAA – 3'.

Metaphase spreads and chromosomal aberrations analysis

Cells were transfected with siRNAs (siSCR and siBRCA1) and incubated for 48hrs. Cells were mock treated or treated with 2-OHE2 or 4-OHE2. Twenty hours after irradiation cells were treated with Colcemid (0.2μg/mL- check concentration) for a further 4hrs, after which cells were trypisinized and Cell pellets were resuspended in hypotonic 75mM KCl for 20 min at 37 °C, followed by fixation for 20 min at 4 °C in freshly prepared Carnoy solution (3:1 v/v methanol/acetic acid). After two additional washes in Carnoy solution, cells were dropped onto pre-warmed wet slides, air-dried at room temperature and aged at room temperature for 7 days. Aged slides were hybridised with whole-chromosome fluorescence-labelled DNA probes (XCP, Whole-Chromosome Probe, MetaSystems) directed to chromosomes 1 (fluorochrome FITC) and chromosome 2 (fluorochrome Texas Red) as per manufacturer's instructions. DNA denaturation (72 °C for 3 min) and hybridisation (37 °C for 8 hrs) were performed using the HYBrite chamber system (Vysis). All chromosomes were counterstained with DAPI (Sigma). Coded slides were viewed with an epi-fluorescence microscope (Axioplan2 imaging MOT, Carl Zeiss), connected to an automated system (Metafer 4 software, MetaSystems), for slide scanning and three-colour image acquisition. Chromosome aberrations were analysed on stored images. All slides were scored blind by the same scorer. All types of aberrations were scored

separately and categorised in simple exchanges (i. e. translocations and dicentrics), either visibly structurally complete or incomplete acentric excess fragments and complex exchanges. No centromere probe was used but centromeres were clearly distinguishable as bright bands under DAPI illumination.

Chromatin Immunoprecipitation (ChIP)

Chromatin was cross-linked using 1.5% formaldehyde for 15 minutes at room temperature. Cells were then washed twice with PBS and collected in 1mL collection buffer [100mM Tris-HCL (pH 9.4) and 100mM DTT]. The cell suspension was incubated on ice for 15 minutes. Cells were then lysed sequentially by resuspension and 5-minute centrifugation (3,000g at 4^{o}C) with 1 mL buffer A (10mM EDTA, 0.5mM EGTA, 10mM HEPES, and 0.25% Triton X-100) and 1mL buffer B (1mM EDTA, 0.5mM EGTA, 10mM HEPES, and 200mM NaCl), and sonication, three times for 10 seconds at maximum settings, in 250 mL lysis buffer (10mM EDTA, 50mM Tris-HCl, 1% SDS, and 0.5% Empigen BB). After 15-minute centrifugation, 10 ML of the supernatant was taken as input and the remainder was diluted 5-fold in immunoprecipitation buffer (2mM EDTA, 100mM NaCl, 20mM Tris-HCl, and 0.5% Triton X-100). This was then subjected to immunoprecipitation overnight with specific antibodies after pre-clearing with pre-immune IgG, 2mg salmon sperm DNA, and 60mL protein A/G Sepharose bead slurry. The precipitate complexes were serially washed with 300mL washing buffer I (2mM EDTA, 20mM Tris-HCl, 1% SDS, 01% Triton X-100, and 150mM NaCl), washing buffer II (2mM EDTA, 20mM Tris-HCl, 1% SDS, 01% Triton X-100, and 250mM NaCl), washing buffer III (1mM EDTA, 10mM Tris-HCl, 1% NP40, 1% deoxycholate, and 0.25M LiCl) and twice with 1mM EDTA and 10mM Tris-HCl. Complexes were removed from the beads through subsequent 15-minute incubations, vortexing, and 5-minute centrifugations with 50 ML of 1% SDS, 0.1 mol/L NaHCO3. Cross-linking was reversed overnight at 65^{o}C and the DNA was purified with QIAquick columns (Qiagen). Antibodies used for ChIP were BRCA1

(Ab-1; Calbiochem) and anti-mouse IgG (DAKO). qPCR was performed on ChIP derived DNA using the primers described previously (qRT-PCR analysis section).

. qPCR mediated quantification results are expressed as fold enrichment over input compared to enrichment of a negative control region, using the following primers targeted to the indicated gene promoters: CYP1A1_ChIP_Fwd: CCG AGT CCT GGT AGG CTG TA, CYP1A1_ChIP_Rev: AGA GAG GGT ACG GGA AGC TC, CYP3A4_ChIP_Fwd: CCT TGG ACT CCC CAG TAA CA, CYP3A4_ChIP_Rev: GGA AGA GGC TTC TCC ACC TT, COMT_ChIP_Fwd: CAG CAG GGC TCC AGT AAG AC, COMT_ChIP_Rev: CTA GTG CCT CGT TCC CAG AG, NQO1_ChIP_Fwd: TGG ACT CTC TTG GGA CGA CT, NQO1_ChIP_Rev: CAG AGG CCT CAA AAA TCT GG , ChIP_NonSpecificRegion_Fwd: GTG GTG GGA GCT ACC AAA GA, ChIP_NonSpecificRegion_Rev: CCT TTT GCT CCG TCC AAT TA.

Ultra-performance-liquid-chromatography-tandem-mass spectrometry (UPLC-MS/MS)

Control (n=8) and BRCA1-depleted (n=8) MCF-10A cell cultures in DMEM-F12 medium were treated with 10 nM Estradiol (E2) for 24 h prior to decanting of the cell-free medium. Preliminary studies confirmed that E2 metabolites were secreted into the medium, with minimal metabolites being detected in cell lysates. To improve quantitative accuracy, deuterium labelled d5-2OHE2 and d5-4OHE2 were added as internal standards at fixed concentrations to media samples and analytical controls prior to extraction. Estrogens were extracted from media samples at pH 4 via a double liquid-liquid diethyl ether solvent extraction. Extracts were evaporated to dryness and derivatised with dansyl chloride to enhance the sensitivity of the estrogens under the positive mode electrospray ionization used for mass spectrometric analysis. Derivatisation was performed in 0.5 mg/ml dansyl chloride in 50:50 acetonitrile:0.1M pH 9 bicarbonate buffer for 10 min at 60°C (Xu et al. 2005). Quantitative analysis was performed on a Waters Acquity ultra-performance liquid

chromatography (UPLC) system coupled to a Waters Quattro Premier XE triple quadrupole mass spectrometer operating under MassLynx software. The mobile phase gradient (water or methanol containing 0.1% formic acid) was optimised to ensure baseline chromatographic separation on a pentafluorophenyl (PFP) 100mm column (Phenomenex, Torrance, CA, USA) which yielded much improved separation compared with conventional C18 based columns, and the accurate identification (using paired ion transitions conforming to fixed ratios) and quantification of the isomeric forms of the hydroxyl-estradiol metabolites. A slow gradient change from 85% to 90% methanol/formic acid (mobile phase B) from 2 to 10 mins was required to achieve baseline separation of 2- and 4-OHE2. Sensitivity and specificity were enhanced by the monitoring of a novel fragmentation transition (m/z 755.1>521.1) which represents the loss of only one dansyl group (at the O-S bond) from the bis-dansylated estrogen derivative. Most published methods employ the m/z 170 fragment for quantification which represents a single isolated dansyl fragment (the derivatising agent) and is thus less characteristic of the target molecule. Samples were quantified against extracted matrix-matched calibration standards using Waters QuanLynx software. Within run repeatability at 0.2 ng/ml media was below 10% (coefficient of variation, n=6) for all compounds. The UPLC method was significantly faster (13 min runtime) than traditional HPLC methods for estrogen analysis. Each culture medium sample was analysed in duplicate (2 x 5ml) and the mean observed metabolite concentration normalized on the basis of the viable cell count in each culture flask (concentration data expressed as ng/ml/10^6 cells). Cultures were prepared and analysed in three independent batches. Statistically significant differences between groups were demonstrated using a paired, two-tail Student's t-Test (n=8), pairing control and BRCA1-depleted cultures within each analytical run.

qRT-PCR analysis

2µg of DNase treated RNA was used for cDNA synthesis with Transcriptor High Fidelity cDNA Synthesis Kit (Roche Applied Science), following the manufacturer's instructions. An identical cDNA synthesis reaction was performed for each sample without the addition of reverse transcriptase to be used as a control to ensure no genomic DNA contamination. qRT-PCR was carried out on ROCHE LightCycler 480, using LightCycler 480 Probes Master Mix (ROCHE) and RealTime Ready Catalog Assays (ROCHE) for each gene (*ACTB, CYP1A1, CYP1A2, CYP3A4, CYP1B1, COMT* and *NQO1*), following the manufacturer's instructions. A matched qRT-PCR reaction was carried out using the RT-ve control for each sample ensuring no genomic DNA contamination.

Isolation and culture or primary breast progenitor cells

Ethical approval was obtained through the Northern Ireland Biobank to collect normal breast tissue following informed consent from women undergoing elective breast reduction or risk-reducing mastectomy. Primary epithelial cells were isolated from fresh tissue by digestion overnight at 37 °C in media containing collagenase-hyaluronidase (10% solution in HuMEC ready media (Life Technologies)). The cell suspension was passed through a 40 µm cell sieve then suspended in HuMEC ready media (minus bovine pituitary extract) plus FGF, heparin and Neurocult SM1 supplement for culture as mammospheres in ultra-low attachment 75 cm^2 flasks for 7 days. Mammosphere cultured cells were then dissociated and plated into Lab-Tek II CC^2 treated chamber slides (Nunc) in the media above.