Data Article

Genomic data on breast cancer transcript profile modulation by 17beta-hydroxysteroid dehydrogenase type 1 and 17-beta-estradiol

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A B S T R A C T

The data presented here are related to the research article entitled “Estradiol-independent modulation of breast cancer transcript profile by 17beta-hydroxysteroid dehydrogenase type 1” (J.A. Aka, E.L. Calvo, S.X. Lin, 2016) [1]. We evaluated the effect of the steroidal enzyme 17β-HSD1 and its product, the estrogenic hormone 17-beta-estradiol (E2), on gene transcription profile of breast cancer cells. RNA interference technique was used to knock down the 17β-HSD1 gene (HSD17B1) in the hormone-dependent breast cancer cell line T47D in steroid-deprived medium. Transfected cells were subsequently treated with E2, and microarray analyses (with three contrasts) were used to investigate (i) the effect of 17β-HSD1 expression on breast cancer cell transcript profile in steroid-deprived condition, (ii) the effect of E2 on breast cancer gene expression and (iii) if E2 affects gene regulation by 17β-HSD1. Functional enrichments of the differentially expressed genes were assessed using Ingenuity Pathway Analysis (IPA). Here, we showed data on 140 genes that are induced or repressed 1.5 time or higher (p < 0.05) in the HSD17B1-silenced and E2-treated T47D cells revealed by microarray analysis, and presented the 14 functional terms found in the cancer and in the cell death and survival
categories revealed by the IPA biological function analysis. Data on IPA Canonical Pathway and network analyses is also presented. Further discussion on gene regulation by 17\(\beta\)-HSD1 and E2 is provided in the accompanying publication [1].

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### Specifications Table

| Subject area       | Biology                                      |
|--------------------|----------------------------------------------|
| More specific subject area | Breast cancer                                |
| Type of data       | Table, Figure                                |
| How data was acquired | Microarray analysis: microarray was processed using Affymetrix GeneChip Whole Transcript (WT) Sense Target Labeling Assay and quantified Affymetrix image files were analyzed using the Bioconductor package OneChannelGUI into the statistical software environment R. Bioinformatics analysis: functional enrichment analysis was done using the gene list from the microarray analysis and Ingenuity pathway core analysis (IPA\(^*\), QIAGEN Redwood City). |
| Data format        | Analyzed and filtered data                    |
| Experimental factors | T47D cells were transfected with 17\(\beta\)-HSD1 siRNAs followed by estradiol treatment two days later for an additional two days, and total RNA was extracted for analysis. |
| Experimental features | 3 \times 10^5 T47D cells were transfected in 6-well plates in charcoal-treated medium with 200 nM mixed 17\(\beta\)-HSD1 specific siRNAs or with negative control siRNA. Two days later, transfected cells were treated with 1 nM estradiol or ethanol as a vehicle control in fresh charcoal-treated medium and cells were incubated for two additional days before RNA extraction and analysis. |
| Data source location | N/A                                           |
| Data accessibility | Data is available within this article and available at the NCBI database via Gene Expression Omnibus (GEO accession number GSE77345). |

### Value of the data

- Provide information on genes regulated by 17\(\beta\)-HSD1 and its product estradiol, useful for further studies on breast cancer cell mechanisms.
- Can contribute to elucidate hormone-independent cell growth pathways in hormone-dependent breast cancer cells.
- May stimulate further research on understanding 17\(\beta\)-HSD1 roles in breast cancer development.

### 1. Data

Table 1 showed data on gene expression profile in T47D cells (genes regulated 1.5 time or higher) transfected with 17\(\beta\)-HSD1 siRNAs (si17B1) or negative control siRNA (NC), and treated with 1 nM
Table 1
List of the 140 genes induced or repressed 1.5 time or higher (p < 0.05) in T47D cells after transfection with 17β-HSD1 siRNAs (si17B1) or negative control siRNA (NC) for two days and cell treatment with 1 nM estradiol (E2). Data was obtained from microarray analysis using contrast NC+E2 vs. si17B1+E2 (see Table 5).

| Symbol | Description | Fold change |
|--------|-------------|-------------|
| CDH10 | Cadherin 10, type 2 (T2-cadherin) | 5.2 |
| IFI44 | Interferon-induced protein 44 | 4.7 |
| IFIT2 | Interferon-induced protein with tetratricopeptide repeats 2 | 4.6 |
| IFIT3 | Interferon-induced protein with tetratricopeptide repeats 3 | 4.4 |
| RSAD2 | Radical S-adenosyl methionine domain containing 2 | 4.3 |
| DXD60L | DEAD (Asp-Glu-Ala-Asp) box polypeptide 60-like | 4.0 |
| CCL5 | Chemokine (C-C motif) ligand 5 | 3.4 |
| IFIT1 | Interferon-induced protein with tetratricopeptide repeats 1 | 3.4 |
| ARL4D | ADP-ribosylation factor-like 4D | 3.3 |
| NAALADL2 | N-acetylated alpha-linked acidic dipeptidase-like 2 | 3.2 |
| BTN3A2 | Butyrophilin, subfamily 3, member A2 | 3.1 |
| LBA1 | Lupus brain antigen 1 | 3.1 |
| DXD60 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 60 | 3.0 |
| XAF1 | XIAP associated factor 1 | 2.9 |
| MDGA2 | MAM domain containing glycosylphosphatidylinositol anchor 2 | 2.9 |
| OAS2 | 2′-5′-oligoadenylate synthetase 2, 69/71 kDa | 2.9 |
| RARR53 | Retinoic acid receptor responder (tazarotene induced) | 2.9 |
| HCP5 | HLA complex P5 | 2.9 |
| OASL | 2′-5′-oligoadenylate synthetase-like | 2.9 |
| PAP14 | Poly (ADP-ribose) polymerase family, member 14 | 2.8 |
| IFITM3 | Interferon induced transmembrane protein 3 (1-8U) | 2.8 |
| LAMP3 | Lyosomal-associated membrane protein 3 | 2.7 |
| AKAP6 | A kinase (PRKA) anchor protein 6 | 2.7 |
| PSMB9 | Proteasome subunit beta type-9 | 2.7 |
| BTN3A1 | Butyrophilin, subfamily 3, member A1 | 2.7 |
| RANBP3L | RAN binding protein 3-like | 2.6 |
| HLA-F | Major histocompatibility complex, class I, F | 2.5 |
| IFITM1 | Interferon induced transmembrane protein 1 (9–27) | 2.5 |
| DXS58 | DEAD (Asp–Glu–Ala–Asp) box polypeptide 58 | 2.5 |
| BTN3A3 | Butyrophilin, subfamily 3, member A3 | 2.5 |
| EBRB4 | V-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian) | 2.5 |
| HLA-H | Major histocompatibility complex, class I, H (pseudogene) | 2.4 |
| HLA-A | Major histocompatibility complex, class I, A | 2.4 |
| PARP9 | Poly (ADP-ribose) polymerase family, member 9 | 2.4 |
| HLA-C | Major histocompatibility complex, class I, C | 2.3 |
| CTSO | Cathepsin O | 2.3 |
| LMBTL3 | L(3)mbt-like 3 (Drosophila) | 2.3 |
| HLA-E | Major histocompatibility complex, class I, E | 2.3 |
| IFI35 | Interferon-induced protein 35 | 2.3 |
| HLA-B | Major histocompatibility complex, class I, B | 2.3 |
| APOL1 | Apolipoprotein L, 1 | 2.3 |
| HLA-G | Major histocompatibility complex, class I, G | 2.3 |
| HERC6 | Hect domain and RLD 6 | 2.2 |
| HERC5 | Hect domain and RLD 5 | 2.2 |
| IFIH1 | Interferon induced with helicase C domain 1 | 2.2 |
| SLC46A3 | Solute carrier family 46, member 3 | 2.2 |
| VGLL1 | Vestigial like 1 (Drosophila) | 2.2 |
| TAP1 | Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) | 2.2 |
| FGF12 | Fibroblast growth factor 12 | 2.2 |
| PSRC1 | Phospholipid scramblase 1 | 2.2 |
| HLA-A29 | Major histocompatibility complex class I HLA-A29 | 2.2 |
| LGALS3BP | Lectin, galactoside-binding, soluble, 3 binding protein | 2.2 |
| SAMD9 | Sterile alpha motif domain containing 9 | 2.2 |
| ATP1B1 | Atpase, Na+/K+ transporting, beta 1 polypeptide | 2.1 |
| OAS1 | 2′-5′-oligoadenylate synthetase 1, 40/46kda | 2.1 |
| PSMB8 | Proteasome subunit beta type-9 | 2.1 |
| OAS3 | 2′-5′-oligoadenylate synthetase 3, 100kda | 2.1 |
| CENTD1 | Centaurin, delta 1 | 2.1 |
Table 1 (continued)

| Symbol     | Description                                                                 | Fold change |
|------------|-----------------------------------------------------------------------------|-------------|
| IFI27      | Interferon, alpha-inducible protein 27                                      | 2.1         |
| DHX58      | DEXH (Asp–Glu–X-His) box polypeptide 58                                     | 2.1         |
| STAT2      | Signal transducer and activator of transcription 2, 113kda                  | 2.0         |
| LAMC1      | Laminin, gamma 1 (formerly LAMB2)                                            | 2.0         |
| THSD7A     | Thrombospondin, type I, domain containing 7A                                | 2.0         |
| TGFβ2      | Transforming growth factor, beta 2                                           | 2.0         |
| USP18      | Ubiquitin specific peptidase 18                                             | 2.0         |
| MX1        | Interferon-induced GTP-binding protein Mx1                                  | 2.0         |
| B2M        | Beta-2-microglobulin                                                        | 2.0         |
| DTX3L      | Deltex 3-like (Drosophila)                                                   | 2.0         |
| ERAP1      | Endoplasmic reticulum aminopeptidase 1                                     | 2.0         |
| SLC15A3    | Solute carrier family 15, member 3                                           | 1.9         |
| CFB        | Complement factor B // complement component 2                               | 1.9         |
| TNFSF10    | Tumor necrosis factor (ligand) superfamily, member 10                       | 1.9         |
| ROBO2      | Roundabout homolog 2                                                        | 1.9         |
| IL1R1      | Interleukin 1 receptor, type 1                                              | 1.9         |
| RAB27B     | RAB27B, member RAS oncogene family                                          | 1.9         |
| RTP4       | Receptor (chemosensory) transporter protein 4                               | 1.9         |
| STAT1      | Signal transducer and activator of transcription 1, 91kda                  | 1.9         |
| CASP4      | Caspase 4, apoptosis-related cysteine peptidase                             | 1.9         |
| INSIG2     | Insulin induced gene 2                                                       | 1.9         |
| DDX2P434B2016 | Similar to hypothetical protein LOC284701                | 1.9         |
| SMARCA1    | Nucleosome-remodeling factor subunit SNF2L                                   | 1.8         |
| LIPH       | Lipase, member H                                                            | 1.8         |
| ZNFX1      | Zinc finger, NFX1-type containing 1                                         | 1.8         |
| UBP1       | Upstream binding protein 1 (LBP-1a)                                         | 1.8         |
| KLF8       | Kruppel-like factor 8                                                        | 1.8         |
| LRRK2      | Leucine-rich repeat kinase 2                                                 | 1.8         |
| LBTP1      | Latent transforming growth factor beta binding protein 1                    | 1.8         |
| EV11       | Ecotropic viral integration site 1                                           | 1.8         |
| FBXO32     | F-box protein 32                                                             | 1.7         |
| BCAS1      | Breast carcinoma amplified sequence 1                                       | 1.7         |
| ALCAM      | Activated leukocyte cell adhesion molecule                                   | 1.7         |
| MTERFD3    | MTERF domain containing 3                                                   | 1.6         |
| MMP16      | Matrix metallopeptidase 12                                                    | 1.6         |
| LOC390345  | Similar to ribosomal protein L10                                            | 1.6         |
| CITED2     | Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 2 | 1.6 |
| CYF4Z2P    | Cytochrome P450, family 4, subfamily Z, polypeptide 2 pseudogene             | 1.6         |
| RNF43      | Ring finger protein 43                                                       | 1.6         |
| ZNF175     | Zinc finger protein 175                                                      | 1.5         |
| SEPP1      | Selenoprotein P, plasma, 1                                                   | 1.5         |
| EPAS1      | Endothelial PAS domain protein 1                                             | 1.5         |
| FAM115A    | Family with sequence similarity 115, member A                               | 1.5         |
| Sema6A     | Semaphorin 6A                                                                | 1.5         |
| NBEA       | Neurobeachin                                                                | 1.5         |
| CSAD       | Cysteine sulfenic acid decarboxylase                                         | 1.5         |
| SNX24      | Sorting nexin 24                                                             | 1.5         |
| RBP8       | Retinoblastoma binding protein 8                                             | 1.5         |
| PTTG1      | Pituitary tumor-transforming 1                                               | 1.5         |
| ELOVL2     | Elongation of very long chain fatty acids protein 2                          | 1.5         |
| KCTD6      | Potassium channel tetramerisation domain containing 6                       | 1.5         |
| AURKA      | Aurora kinase A                                                             | 1.5         |
| KIF18A     | Kinesin family member 18A                                                   | 1.5         |
| ARHGAP11A  | Rho GTPase activating protein 11A                                            | 1.5         |
| CDK9N3     | Cyclin-dependent kinase inhibitor 3                                          | 1.5         |
| PRR11      | Proline rich 11                                                              | 1.5         |
| C13orf3    | Chromosome 13 open reading frame 3                                           | 1.5         |
| SPAG5      | Sperm associated antigen 5                                                   | 1.5         |
| CA8        | Carbonic anhydrase VIII                                                      | 1.6         |
| PLK1       | Polo-like kinase 1 (Drosophila)                                              | 1.6         |
| SGOL1      | Shugoshin-like 1 (S. pombe)                                                  | 1.6         |
estradiol (E2), revealed by microarray analysis with contrast NC+E2 vs. si17B1+E2 (see Table 5 for additional information). Table 2 showed the 14 functional terms found in the cancer category of the IPA biological function analysis of 208 genes from three fold change lists (genes which fold change equal or higher than 1.5 in at least one contrast), generated by the three contrasts (NC vs. si17B1; NC vs. NC+E2; and NC+E2 vs. si17B1+E2) of microarray analyses (see Table 5 for contrast description). Table 3 showed the 14 functional terms found in the cell death and survival category of the IPA biological function analysis of 208 genes generated by the three contrasts of microarray analyses. Table 4 showed data on the IPA network analysis of 208 genes from the three contrasts (NC vs. si17B1;
NC vs. NC+E2; and NC+E2 vs. si17B1+E2) of the microarray analysis. Figs. 1–4 showed IPA Canonical pathway analyses for interferon signaling pathway in the NC vs. NC+E2 contrast (Fig. 1), antigen presentation pathway in the NC vs. si17B1 contrast (Fig. 2), antigen presentation pathway in the NC vs. NC+E2 contrast (Fig. 3), and role of BRCA1 in DNA damage response in the NC vs. NC+E2 contrast (Fig. 4).

2. Experimental design, materials and methods

2.1. Cell culture, siRNA transfections, steroid treatment and RNA preparation

T47D cells were obtained from the American Type Culture Collection (ATCC) and were cultured as described in ref [1]. The detailed procedure of siRNA transfections, steroid treatment and RNA preparation have been described in ref [1]. Briefly, two days before transfection, T47D cells were cultured in dextran-coated charcoal-treated medium; on the transfection day, 3 × 10^5 cells were reverse-transfected in 6-well plates with 200 nM mixed 17β-HSD1 specific siRNAs [2,3] (si17B1) or with Scramble siRNA used as negative control siRNA (NC) using Lipofectamine siRNA Max (Invitrogen), and cells were incubated in steroid deprived medium. Two days after transfection, cell culture media were replaced by fresh charcoal-treated medium containing either the steroid estradiol (1 nM) or ethanol as a vehicle control (see Table 5), and cells were incubated for two more days before RNA extraction using Trizol Reagent (Invitrogen). The RNA samples included two independent biological replicates, coming from two independent cell culture experiments, for a total of eight RNA samples.

2.2. Microarray processing

RNA samples were processed according to the manufacturer’s recommended procedures on GeneChip Whole Transcript (WT) Sense Target Labeling Assay from Affymetrix (http://www.affymetrix.com/support/downloads/manuals/wt_sensetarget_label_manual.pdf). The assay was started with 0.2 μg of each T47D cells RNA samples and the protocol is based on the principle of performing one cycle of cDNA synthesis and in vitro transcription (IVT) for target amplification to generate cRNA following by reverse transcription reactions to synthesis the WT cDNA. About 2.7 μg sample of

| Number | Functional term |
|--------|-----------------|
| 1      | Apoptosis       |
| 2      | Apoptosis of breast cancer cell lines |
| 3      | Apoptosis of breast cell lines |
| 4      | Apoptosis of mammary epithelial cells |
| 5      | Apoptosis of mammary tumor cells |
| 6      | Apoptosis of tumor cell lines |
| 7      | Cell death       |
| 8      | Cell death of tumor cell lines |
| 9      | Cell survival    |
| 10     | Cell viability   |
| 11     | Cytotoxicity of cells |
| 12     | Cytotoxicity of cytotoxic T cells |
| 13     | Cytotoxicity of T lymphocytes |
| 14     | Necrosis         |

Table 3
The 14 functional terms found in the cell death and survival category of the IPA Biological function analysis of 208 genes regulated by 17β-HSD1 and/or estradiol in T47D cells.
Table 4
IPA network analysis of 208 genes regulated by 17β-HSD1 and/or estradiol in T47D cells from the three contrasts listed in Table 5.

Molecules in Network: All of the molecules that compose each network are listed.

Score: The score is based on a p-value calculation, which calculates the likelihood that the Network Eligible Molecules that are part of a network are found therein by random chance alone. Mathematically, the score is simply the negative exponent of the right-tailed Fisher’s exact test result. For example, if the score is 3, then the there is a 1 in 1000 chance that the Network Eligible Molecules found in that network appeared there just by chance. In other words, the score is simply a measure of the number of Network Eligible Molecules in a network, and the greater the number of Network Eligible Molecules in a network, the higher the score (lower the p-value) will be.

Focus Molecules: This column simply indicates the number of Network Eligible Molecules per network. Since the maximum number of molecules per network is currently limited to 35, the number of Network Eligible Molecules per network cannot exceed 35.

Top Functions: Only the three most significant functions for each network are listed.

| ID | Molecules in network | Score | Focus molecules | Top functions |
|----|----------------------|-------|----------------|--------------|
| 1  | Z’ 5’ oas, Akt, DDX58, DDX60, DHX58, FBX032, Fcer1, HERC5, IFIT1, IFIT3, IFITM1, IFITM3, Ifn, IFN Beta, Interferon alpha, IRF, MX1, N-cor, OAS1, OAS2, OAS3, Oas, PARP9, PBK, PI3K (family), RARRES3, RCN2, RSAD2, SEMA6A, STAT2, STAT-1/2, Thioredoxin reductase, TXNRD1, USP18, VTCN1 | 42    | 23             | Antimicrobial response, inflammatory response, inflammatory disease |
| 2  | Alpha catenin, Alpha tubulin, AREG/AREGB, Cadherin, CDH10, Cg, CITED2, EPAS1, ERBB2, ERBB4, estrogen receptor, FKBp4, GNMT, GREB1, Hdac, Histone h3, Hsp70, Hsp90, ID1, MCM10, PKFB3, PGR, Pkcs, POLE3, PTGES, RNA polymerase II, RPL22, SMARC1, SPINK4, STC2, STEAP2, TCF, TM4SF1, Ubiquitin | 38    | 22             | Connective tissue development and function, embryonic development, organ development |
| 3  | androstenediol, AR44, CSAD, CD97, CSAD, CYP1A1, EID3, ESRI, FLRT3, HLA-C, ICAM3, IL6, KCM1, KCHN1, KCTD6, L3MBTL3, LRRK2, mir-19, miR-149-3p (and other miRNAs w/seed GCGAGGC), miR-183-5p (miRNAs w/seed AUUGCCAC), miR-19b-3p (and other miRNAs w/seed GUGCAAA), MTERFD3, NAALADL2, NMU, RAB27B, RAPGEF1, RCN2, RNF135, SLC46A3, SLC47A1, SLC19a1, SOX13, SPC25, THSD7A, TMEM116 | 38    | 21             | Organismal injury and abnormalities, reproductive system development and function, reproductive system disease |
| 4  | ADRB, APC (complex), AURKA, BRCA2, BUB1, calpain, CASP4, caspase, CCNA2, CCNB2, Cdc2, CDC20, Cdk, CKS2, Cyclin A, Cyclin B, Cyclin D, Cyclin E, E2F, FLNA, Ifn gamma, MAP2K1/2, NDC80, NFkB (complex), NFKB, NUP2, PLK1, PP2A, PTG1, RAD51, Rb, RHD2D2A, SGO1, SPAG5, XAF1 | 33    | 19             | Cell cycle, cellular assembly and organization, DNA replication, recombination, and repair |
| 5  | AKAP81, ANP32E, APEH, ARHGPAP11A, BTN3A1, CEP152, DTX3L, FAM115A, FBX038, FDP5, FTSJ3, HMG CoA synthase, IFRAD2, INS2G2, KLF8, LGALS7/LGALS7B, LRRC41, MDGA2, NBEA, OAS3, PABPC4, PARP9, PHF7, PPM1G, PRR11, PXMP4, RBM24, RHOGTB1, RNASEH2B, RNFL23, SKA3, SNRP4, SPC24, UBC, ZNF622 | 30    | 18             | Hereditary disorder, neurological disease, psychological disorders |
| 6  | 20S proteasome, B2M, CD8, ERAP1, ERK1/2, H-2db, HLA Class I, HLA-A, Hla-abc, HLA-B27, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, IFI27, IFI35, IFI44, IFIH1, IFIT2, IFN alpha/beta, IFN type 1, Interferon-Î± Induced, ISGF3, KIR, LGALS3BP, MHC, MHC Class I (complex), MHC CLASS I (family), MHC I-Î±, PSMB8, PSMB9, Stat1-Stat2, TAP1, Tap | 28    | 17             | Endocrine system disorders, gastrointestinal disease, immunological disease |
| Gene Symbols | Relevant Phenotypes |
|-------------|---------------------|
| AHNAK2, ANKRD27, ARL8B, BRCA2, BTN3A3, CA8, CBX8, CEP170, CKAP2L, CPAMD8, CTS1L, DDIX6L, DLG5, DNPEP, ESCO2, EXOC1, FAM72A, GOLGA4, HCST, HIC1, KIF18A, KIF20A, KIF4A, LIPH, LPXN, MICB, PSMD14, RAB6A, RAB6B, SNX24, TAZ, TUBCCF2, UBC, ZNF175, ZRANB2 | Connective tissue disorders, dermatological diseases and conditions, developmental disorder |
| 26s Proteasome, Actin, ARAP2, BTN3A2, C8orf44-SGK3/SGK3, FGF12, GN1, GN1G1, GSK3B, GSKIP, HCP5, IFI35, IFNC, IFNL3, IL19, IRF, Irgm, Lamp3, mir-21-5p (and other miRNAs w/seed AGCUUAU), Mov10, Mtorc2, Oas, Pla2, Psmb9, Psme2, Rbnp3l, Rsad2, Rtp4, Samd9, Slc15a3, Slc9a3, Soxs, Stat, uric acid, Usp18 | Dermatological diseases and conditions, infectious disease, cell-to-cell signaling and interaction |
| AKAP6, ALCAM, Apol1, Cdkn3, Creb2f, Ctsd, Ddit4, Fbp1, Fsh, Gsk3, Hemoglobin, Histone h4, Ikk (complex), Insulin, Kcnma1, Lh, Mapk, Myc, Notch, Oasl, P85 (pik3r), Pka, Prikac, Rac, Ras, Ras homolog, Robo2, Selenbp1, Shc, Sox2, Tcr, Trip13, Vegf, Znf1x1 | Cellular growth and proliferation, tissue development, cell morphology |
| Alp, Ap1, Cbp/p300, Ccl5, Cdc42, Collagen type I, Collagen type IV, Collagen(s), Elov2, Erk, Focal adhesion kinase, Hey2, Il1r1, Integrin, Jak, Kyn, Laminin, Laminin, LdL, Ltbp1, Mek, Myb, p70 S6k, Pdgf (complex), Pdgf Bb, Pias, Pka, Smad, Smad2/3, Sos, Stat5a/b, Tgf beta, Tgfb2, Thbs1 | Cardiovascular disease, embryonic development, organ development |
| Alpha-estriadiol, Androstenediol, Atf12a, Atf1b1, Bcas1, Beta-estriadiol, Cd40, Clk2, Ctscl, Egfr, Egfr ligand, Egfr-Erb2, Erbb, Ganglioside Gd1a, Grm4, Ier2, If30, Ifne, Ifg, Ltb, mir-146, mir-29b-3p (and other miRNAs w/seed AGCACCA), Mmp, Ofm1, Ptgd, Pvrl4, Rac1, Rerg, Sepp1, SerpinA6, Tap1, Tp53inp1, Ubp1, Vgll1, Wap | Cell morphology, cellular assembly and organization, cellular development |
| Ampk, Bcr, Cdk, Cytoskeleton C, F Actin, HerC6, Hsp27, Ige, IgG1, IgG, Llm, Lkk (family), Il1, Il12 (complex), Il12 (family), Immunoglobulin, Jnk, Mecom, Mhc class II (complex), Mybl1, P38 Mapk, Parp14, Parp, Pt3k (complex), Pdk gamma, Plscr1, Pro-inflammatory Cytokine, Rsk, Rxr, S100A8, Src (family), Stat1, Tlr, Tnf (family), Tnfsf10 | Cellular development, hematological system development and function, hematopoiesis |
fragmented cDNAs was used to hybridize human oligonucleotide array Gene 1.0 ST (Genechip; Affymetrix). The array comprised more than 750,000 unique 25-mer oligonucleotides constituting over 28,000 gene-level probe sets of the human genome. The cDNA probe corresponding to each biological replication for each condition was hybridized on separate arrays. After hybridization, chips were processed using the Affymetrix GeneChip Fluidic Station 450 (protocol F450_0007). Chips were scanned with a GeneChip scanner 3000 7G (Affymetrix) and images were extracted with the

Table 5
Summary of the cell experiments and microarray analyses.

| Time  | Experiments                                      | Well 1   | Well 2   | Well 3   | Well 4   |
|-------|--------------------------------------------------|----------|----------|----------|----------|
| Day 1 | Transfection with negative control (NC) or 17β-HSD1 (si17B1) siRNAs | NC       | si17B1   | NC       | si17B1   |
| Day 3 | Addition of estradiol (+E2) or the vehicle control (−) | −        | −        | +E2      | +E2      |
| Day 5 | Cell wash and total RNA extraction               | NC       | si17B1   | NC+E2    | si17B1+E2|

Microarray analyses
Three contrasts
- Contrast 1: NC vs. si17B1
- Contrast 2: NC vs. NC+E2
- Contrast 3: NC+E2 vs. si17B1+E2

Aim
- List T47D genes impacted by 17β-HSD1 knockdown in steroid-deprived medium
- List genes responsive to estrogen in T47D
- To detect if E2 impacts gene regulation by 17β-HSD1 knockdown

Fig. 1. IPA Canonical Pathway analysis showing the interferon signaling pathway across the NC vs. NC+E2 contrast data.
GeneChip operating software (Affymetrix GCOS v1.4). The microarray processing was performed at the DNA Biochip Platform service at CHU de Québec - CHUL Research Centre (Québec, Canada).

2.3. Microarray analysis

The microarray analysis has been described in the accompanying paper [1]. Quantified Affymetrix image files ("CEL" files) for each of the treatment conditions (including two independent replicates per treatment condition) were used to perform the microarray analyses using the Bioconductor package OneChannelGUI [4,5] in the statistical software environment R. Three contrasts (see Table 5) were using the RMA method [6]. Data filtering was performed at signal feature level by interquantile range (IQR) then by intensity. To identify differentially expressed genes, gene expression intensity was compared using a moderated t-test and a Bayes smoothing approach developed for a low number of replicates [7], and the false discovery rate was estimated from P-values derived from the moderated t-test statistics for correction for the effect of multiple testing [8]. Genes were considered to be significantly differentially expressed if p-values were < 0.05. The log₂ transformed signal intensities

Fig. 2. Canonical pathways by IPA: antigen presentation pathway in the NC vs. si17B1 contrast.
were averaged, and the mean value was used to compute the fold changes. Genes that were differentially expressed 1.5-fold or higher were considered for subsequent analyses. Our microarray data is available in the Gene Expression Omnibus (GEO) repository, accession number GSE77345.

2.4. Functional enrichment analysis

Ingenuity pathway analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity) was used to assess the functional enrichment of the 208 modulated genes revealed by the three-contrast microarray analysis (genes which fold change equal or higher than 1.5 in at least one contrast). Three analyses made by IPA were presented here: identification of biological functions, gene networks and canonical pathways (see ref for additional information). Criteria used for the IPA analyses have been described in the accompanying research article [1].
Fig. 4. Canonical pathways by IPA: role of BRCA1 in DNA damage response in the NC vs. NC+E2 contrast.

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