Comparison of Functional Proteomic Analyses of Human Breast Cancer Cell Lines T47D and MCF7

Juliette Adjo Aka¹,², Sheng-Xiang Lin¹,²*

¹Laboratory of Endocrinology and Genomics, CHUL Research Center (CHUQ), Laval University, Quebec City, Quebec, Canada, ²Department of Molecular Medicine, Faculty of Medicine, Laval University, Quebec City, Quebec, Canada

Abstract

T47D and MCF7 are two human hormone-dependent breast cancer cell lines which are widely used as experimental models for in vitro and in vivo (tumor xenografts) breast cancer studies. Several proteins involved in cancer development were identified in these cell lines by proteomic analyses. Although these studies reported the proteomic profiles of each cell line, until now, their differential protein expression profiles have not been established. Here, we used two-dimensional gel and mass spectrometry analyses to compare the proteomic profiles of the two cell lines, T47D and MCF7. Our data revealed that more than 164 proteins are differentially expressed between them. According to their biological functions, the results showed that proteins involved in cell growth stimulation, anti-apoptosis mechanisms and cancerogenesis are more strongly expressed in T47D than in MCF7. These proteins include G1/S-specific cyclin-D3 and prohibitin. Proteins implicated in transcription repression and apoptosis regulation, including transcriptional repressor NF-X1, nitrilase homolog 2 and interleukin-10, are, on the contrary, more strongly expressed in MCF7 as compared to T47D. Five proteins that were previously described as breast cancer biomarkers, namely cathepsin D, cathepsin B, protein S100-A14, heat shock protein beta-1 (HSP27) and proliferating cell nuclear antigen (PCNA), are found to be differentially expressed in the two cell lines. A list of differentially expressed proteins between T47D and MCF7 was generated, providing useful information for further studies of breast cancer mechanisms with these cell lines as models.

Introduction

Breast cancer is the most frequent cancer affecting women. The malignancy accounts for about 1 in 10 cancers in the world and is diagnosed in one million women each year [1,2]. In North America (United States and Canada), breast cancer is the second most frequent cause of cancer death in women, after lung cancer, and the leading cause of cancer death among those aged 20–59 years old [3,4]. After increasing through the 80 s and 90 s, breast cancer incidence rates fortunately decreased by 3.5% per year until 2001 to 2004 and the mortality rate decreased by 1.9% per year in the United States between 1998 and 2006 [3,4]. This reflects an improvement in the diagnosis and treatment of the disease, but this cancer nonetheless remains of prime importance.

Human breast cancer cell lines provide an excellent platform for breast cancer research in tumor progression and treatment. T47D and MCF7 are two human hormone-dependent breast cancer cell lines which are widely used as experimental models for breast cancer studies. The two cell lines were generally used for both the in vitro (in cell culture) and in vivo (tumor xenograft in nude mice) analyses of gene and protein function and inhibitor efficacy assessment [5–7]. They were both originally derived from a metastatic site of pleural effusion (ATCC, www.atcc.org) and express estrogen receptors. Several proteins and enzymes that are involved in cell proliferation and in cancer development were identified in these cell lines by proteomic studies [8–10]. Although these studies reported the proteomic profiles of each of these cell lines, until now, no study had established their differential protein expression profile. Using a proteomic approach including two-dimensional (2-D) gel electrophoresis and mass spectrometry (MS) analyses, we establish here the proteomic differences between the T47D and MCF7 cell lines.

Methods

Cell culture

T47D and MCF7 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA). MCF7 cells were maintained in DME low glucose medium supplemented with 1 nM β-estradiol (β-E2). T47D cells were propagated in DME high glucose medium containing 7.5 mg/L bovine insulin (Sigma, Oakville, Ontario, Canada). Both cell types were cultured in phenol red-free media containing 10% fetal bovine serum (FBS) and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO₂.

Generation of protein extracts for proteomics analysis

MCF7 and T47D cells were cultured in T75 flasks in complete growth medium. After three passages, cells were plated in 100×2 cm² dishes and cultured until reaching 80–90%
confluence. Cells were washed two times with cold PBS 1 x, scraped with a policeman in 1.2 mL PBS, collected in an eppendorf and centrifuged at 3000 rpm for 5 min. The cell pellets were resuspended in 500 μl lysis buffer T8 (7 M urea, 2 M thiourea, 3% CHAPS, 20 mM DTT, 5 mM TCEP, 0.5% IPG buffer pH 4–7, 0.25% IPG buffer pH 3–10) containing 50 mM tris-HCl pH 8.8, 1 mM PMSF and 1% protease inhibitors cocktail (EMD Chemicals, Gibbs-town, NJ). Protein samples were precipitated using 2-D Clean-Up Kit (GE Healthcare, Piscataway, NJ) and resolubilized in T8 buffer. Protein samples included three independent biological replicates (coming from three independent cell culture experiments), representing total proteins from each cell line (MCF7 and T47D) for a total of six samples. The protein concentrations were determined using the 2-D Quant Kit (GE Healthcare).

Two-dimensional gel electrophoresis

For the first dimension, 200 μg total protein samples from MCF7 and T47D cell lines were loaded onto 24-cm pH 4–7 Immobilized pH gradient (IPG) strips (Immobiline DryStrips; GE Healthcare). Strips were rehydrated for 10 hours at 30 volts and isoelectric focusing was performed on an IPGphorII IEF system (GE Healthcare). For the second-dimension SDS-PAGE, focused Immobiline DryStrips were equilibrated twice for 15 min in an equilibration buffer (50 mM tris-HCl pH 8.8, 6 M urea, 30% glycerol, 2% SDS, trace of bromophenol blue) containing 10 mg/mL DTT for the first equilibration and 25 mg/mL iodoacetamide for the second one. Immobiline DryStrips were then transferred onto the surface of a 12% acrylamide gel and sealed using 0.5% agarose. Gels were run in Ettan DALT twelve system (GE Healthcare) in a standard tris-glycine SDS-PAGE buffer at 40 mA/gel and 15°C until the tracking dye reached the end of the gel. Three independent protein samples coming from three independent cell culture experiments were run for each cell line. Gels were fixed overnight in 40% methanol, 7% acetic acid, stained with Sypro Ruby (Invitrogen, Burlington, Ontario, Canada) and scanned with the ProXpress CCD scanner (PerkinElmer, Waltham, MA). The 2-D gel electrophoresis was performed at the Proteomic platform of the Infectious Disease Research Center (Quebec, Canada).

Two-dimensional gel image analysis

Protein spot detection, spot matching and semiquantitative statistical analysis were performed using the Progenesis software version PG240 (Nonlinear Dynamics, Durham, NC). For each cell line, three different gel images were analysed and a corresponding synthetic image reference was obtained. After computer matching, detected spots and spot matches were manually edited for more

Figure 1. Proteomic analysis of T47D and MCF7 cells using 2-D gels and mass spectrometry. (A) Representative 2-D gel images for T47D and MCF7 cells showing some differentially expressed spots. The 2-D gels were scanned and the differentially expressed (2-fold or higher, p<0.05) spots were detected using Progenesis software. Arrows indicate some identified protein spots picked for MS analysis. The numbers refer to the spot number listed in Table 1 and 2. Spot numbers 2685, 2879 and 4364 were unique in either cell line whereas spot numbers 2903 and 4375 were up-regulated in one cell line compared to the other cell line. 1 and 2 represent two different regions of the entire 2-D gel images. (B) Summary of the numbers of spots and proteins obtained from the proteomics data

| Total number spots | Variant spots (>2-fold) | Number of unique spots | Spots picked for MS | Proteins identified | Distinct proteins |
|---------------------|------------------------|------------------------|---------------------|--------------------|------------------|
| T47D 3430           | 70 (31 up, 39 down)*   | 12                     | 8 (unique)          | 58                 | 52               |
| MCF7 3132           |                        | 15                     | 8 (unique)          | 22                 | 16               |

* Up-regulated (up) and down-regulated (down) proteins in T47D cell line as compared to MCF7 cell line.

doi:10.1371/journal.pone.0031532.g001
### Table 1. Mass spectrometry identification of proteins in spots up-regulated in or unique to T47D as compared to MCF7 cell line.

| Spot | FC  | Description                                      | UniProt number | MW exp/pred (kDa) | pl exp | Pep | Function description and/or biological process                                      |
|------|-----|--------------------------------------------------|----------------|-------------------|-------|-----|--------------------------------------------------------------------------------------|
| 2810 | 3.0 | Phosphoserine phosphatase                        | P78330         | 26/25             | 5.4   | 15  | Serine biosynthesis                                                                  |
|      |     | Uncharacterized protein EIF4E (Fragment)         | A8M7X2         | 26/25             | 5.4   | 5   | Protein biosynthesis                                                                 |
|      |     | cDNA FLJ76387…arginine/serine-rich 9…           | A8KS3M9        | 26/26             | 5.4   | 5   | Nucleic acid binding                                                                |
|      |     | Transmembrane emp24 domain-containing prot. 7    | Q9Y3B3         | 26/26             | 5.4   | 5   | Transport                                                                           |
|      |     | Heat shock protein beta-1†                      | P04792         | 26/23             | 5.4   | 3   | Stress resistance, actin organization                                               |
|      |     | Peroxiredoxin-4                                 | Q13162         | 26/31             | 5.4   | 2   | Redox regulation of the cell                                                        |
| 2490 | 6.1 | P09493-3 Isoform 3 of P09493                    | P09493-3       | 32/33             | 4.6   | 38  | Muscle contraction                                                                  |
|      |     | Proliferating cell nuclear antigen±             | P12004         | 32/29             | 4.6   | 16  | DNA replication                                                                    |
| 2682 | 2.1 | Protein CDV3 homolog*                           | Q9UKY7         | 28/27             | 5.4   | 6   | Cell proliferation                                                                  |
|      |     | N(G,N(G)-dimethylarginine dimethylaminohydrolase 2 | Q95865   | 28/30             | 5.4   | 5   | Anti-apoptosis, arginine catabolism                                                 |
|      |     | Prohibitin                                       | P35232         | 28/30             | 5.4   | 5   | DNA synthesis, cell proliferation                                                   |
| 2869 | 3.2 | Glutathione 5-transferease Mu 2                 | P28161         | 25/26             | 6.1   | 53  | Metabolic process                                                                   |
|      |     | 5’(3’)-deoxyribonucleotidase, cytosolic type     | Q8TCD5         | 25/23             | 6.1   | 25  | Nucleotide metabolism                                                               |
|      |     | 17β-Hydroxysteroid dehydrogenase 10*            | Q5H927         | 25/26             | 6.1   | 6   | Oxidoreductase - metabolism                                                        |
|      |     | Protein-L-isocitrate(L-aspartate) O-methyltransferase | P22061    | 25/25             | 6.1   | 4   | Protein methylation, protein repair                                                  |
| 2903 | 4.3 | Cathepsin B†                                     | P07858         | 24/38             | 5.1   | 4   | Tumor invasion and metastasis                                                      |
|      |     | Heat shock protein 75 kDa, mitochondrial         | Q12931         | 24/80             | 5.1   | 3   | Chaperone                                                                          |
| 2399 | 3.0 | Nicotinate-nucleotide pyrophosphorylase          | Q15274         | 35/31             | 5.8   | 9   | Pyridine nucleotide biosynthesis                                                   |
|      |     | Malate dehydrogenase, cytoplasmic               | P40925         | 35/36             | 5.8   | 4   | Gluconeogenesis                                                                    |
|      |     | L-lactate dehydrogenase A chain                 | P00338         | 35/37             | 5.8   | 4   | Glycolysis                                                                         |
|      |     | Farnesyltransferase                             | O95749         | 35/35             | 5.8   | 4   | Isoprene biosynthesis                                                               |
|      |     | Splicing factor, arginine/serine-rich 1         | Q07955         | 35/28             | 5.8   | 3   | mRNA processing                                                                    |
|      |     | STI1 homology and U box-containing protein 1     | Q5H927         | 35/35             | 5.8   | 3   | Chaperone activity modulation                                                      |
|      |     | Heterogeneous nuclear ribonucleoproteins A2/B1   | P22626         | 35/37             | 5.8   | 3   | Pre-mRNA processinghttp://www.uniprot.org/keywords/KW-0508                          |
| 39S  |     | riosomal protein L3, mitochondrial               | P09001         | 35/39             | 5.8   | 2   | Translation                                                                        |
| 2685 | U   | Cathepsin D†                                     | P07339         | 28/45             | 5.0   | 20  | Proteolysis                                                                        |
|      |     | cDNA, FLJ92320, highly similar to Homo sapiens   | B2R533         | 28/28             | 5.0   | 5   | Transferase                                                                        |
|      |     | glutathione 5-transferease theta 2 (GST2), mRNA  | A8M7X2         | 28/28             | 5.0   | 4   | Proteosome activation                                                              |
|      |     | Uncharacterized protein PSME2                    | P29218         | 28/30             | 5.0   | 5   | Phosphatidylinositol biosynthesis                                                  |
|      |     | Chloride intracellular channel protein 1         | Q00299         | 28/27             | 5.0   | 3   | Ion transport                                                                       |
|      |     | Density-regulated protein                        | O43583         | 28/22             | 5.0   | 2   | Translation initiation                                                             |
| 2879 | U   | Heat shock 70 kDa protein 1 (HSP70.1)*           | P08107         | 25/70             | 5.2   | 4   | Stress response, polypeptide folding                                              |
|      |     | A-kinase anchor protein 3                        | Q75969         | 25/95             | 5.2   | 2   | Capacitation and acrosome reaction                                                |
| 2508 | U   | Caspase-3 subunit p12                            | P42574         | 32/32             | 6.2   | 14  | Apoptosis                                                                          |
|      |     | Nuclear protein Hcc-1                           | P82979         | 32/24             | 6.2   | 5   | Cell growth, carcinogenesis                                                       |
|      |     | Guanine nucleotide-binding protein subunit       | P63244         | 32/35             | 6.2   | 4   | Regulation of the activity of kinases, tissue differentiation                      |
|      |     | beta-2-like 1                                   |               |                   |       |     |                                                                                     |
|      |     | G1/S-specific cyclin-D3                        | P30281         | 32/33             | 6.2   | 4   | Cell cycle control                                                                 |
|      |     | ATP synthase subunit gamma, mitochondrial        | P36542         | 32/33             | 6.2   | 3   | ATP synthase, ion transport, transport                                            |
|      |     | Carbonyl reductase [NADPH] 1                     | P16152         | 32/30             | 6.2   | 2   | Catalysis of reduction                                                             |
|      |     | Electron transfer flavoprotein subunit alpha,   | P13804         | 32/35             | 6.2   | 2   | Electron transport                                                                 |
|      |     | mitochondrial                                    |               |                   |       |     |                                                                                     |
|      |     | Voltage-dependent anion-selective channel protein 1 | P21796 | 32/31             | 6.2   | 2   | Apoptosis, host-virus interaction, transport                                       |
|      |     | Pyrroline-5-carboxylate reductase                |               |                   |       |     |                                                                                     |
|      |     | Cell division control protein 2 homolog          | P06493         | 32/34             | 6.2   | 2   | Cell cycle control (s-phase, mitosis)                                             |
|      |     | 2,4-dienoyl-CoA reductase, mitochondrial        | Q16698         | 32/36             | 6.2   | 2   | Oxidoreductase                                                                     |
|      |     | Elongation factor Ts, mitochondrial              | P43897         | 32/35             | 6.2   | 2   | Protein biosynthesis                                                               |
|      |     | cDNA FLJ76011, highly similar to Homo sapiens    | P2508         | 32/33             | 6.2   | 2   | Hydrolase activity                                                                |
Table 1. Cont.

| Spot | FC | exp | pI | MW | Pep | U | exp, pI, MW, or Pep | Function/Protein Name | Subcellular Location | NL | Notes |
|------|----|-----|----|----|-----|---|-----------------|----------------------|---------------------|----|-------|
|      |    |     |    |    |     |   |                 |                      |                     |    |       |

The function description and/or biological process were from the UniProt database (www.uniprot.org). Spot, spot number; FC, fold change; MW, molecular weight; pI, isoelectric point as determined from the 2-D gel experiments; Pep, number of unique peptides; U, unique. The number after the protein name indicated the additional spot in which the protein was found.

1Proteins previously reported to be used as breast cancer biomarkers and which were overexpressed in cancerous cells based on data from the literature [10,14,15].

*Proteins used for Q-RT-PCR validation.

doi:10.1371/journal.pone.0031532.t001

accuracy. A spot had to be present in at least two of the three replicate gels to be considered in the analysis. The detection of protein spots differentially expressed was performed using the t-test and INCA volume and proteins that were differentially expressed 2-fold or higher were considered significant. 40 protein spots that were differentially expressed were selected and were excised from Sypro Ruby-stained 2-D gels using a ProXcision robot (PerkinElmer) and sent for MS analysis.

Mass spectrometry and protein identification

MS experiments were performed by the Proteomics platform of the Eastern Quebec Genomics Center (Quebec, Canada). Protein spots were washed with water and tryptic digestion was performed on a MassPrep liquid handling robot (Waters, Milford, MA) according to the manufacturer's specifications and to the protocol of Shevchenko et al [11] with the modifications suggested by Havlis et al [12]. Peptide samples (an aliquot of the digested proteins) were separated by online reversed-phase (RP) nanoscale capillary liquid chromatography (nanoLC) and analyzed by electrospray mass spectrometry (ES MS/MS). The experiments were performed with a Thermo Surveyor MS pump connected to a LTQ linear ion trap mass spectrometer (ThermoFisher, San Jose, CA) equipped with a nanoelectrospray ion source (ThermoFisher). Peptide separation took place on a PicoFrit column BioBasic C18, 10 cm x 0.075 mm internal diameter (New Objective, Woburn, MA) with a linear gradient from 2-50% solvent B [acetonitrile, 0.1% formic acid] in 30 minutes, at 200 nL/min (obtained by flow-splitting). Mass spectra were acquired using a data dependent acquisition mode using Xcalibur software version 2.0. Each full scan mass spectrum (400 to 2000 m/z) was followed by collision-induced dissociation of the seven most intense ions. The dynamic exclusion (30 sec exclusion duration) function was enabled, and the relative collisional fragmentation energy was set to 35%.

All MS/MS samples were analyzed using Mascot algorithm (Matrix Science, London, UK; version Mascot) and the Uni-ref100_14_0_Homo_sapiens_9606 database (version with 89892 entries). Mascot was searched with a fragment ion mass tolerance of 0.075 Da and a parent ion tolerance of 2.0 Da. Iodoacetamide derivative of cysteine was specified as a fixed modification and oxidation of methionine was specified as a variable modification. Two missed cleavages were allowed. Scaffold (version Scaffold_2_01_02, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. The protein identification cut off was set at a confidence level of 95% (MASCOT score >33) with at least two peptides matching to a protein. Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

Functional analysis of the identified proteins

From each spot, only proteins identified with a probably higher than 95% and with at least two matched unique peptides were considered in the analysis, except for the proteins keratins which were not considered for the analysis. The experimental molecular weight and isoelectric point of each identified protein were determined based on the location of the original spot on the 2-D gel using the Progenesis software. The Uniprot data base (www.uniprot.org) was used to search the function/biological process and the subcellular location of each identified protein. Search in the literature (Pulmed) was used when necessary to complete the information about the function and subcellular location.

Quantitative real-time RT-PCR

Total RNAs were isolated from T47D and MCF7 cells using Trizol Reagent (Invitrogen) in 6-well plates and treated with DNase 1. RNA samples for Q-RT-PCR analyses comprised two biological repetitions for each cell line. The measures of mRNA levels of genes were carried out as previously described [7,13] with Actb, Hprt1 and G6PD genes used as internal controls. The procedures were performed at the Q_RTPCR Platform service at CHUQ-CHUL Research Center (Quebec, Canada). The mRNA levels were expressed as thousand of mRNA copies/µg total RNA and SDs were <10% of duplicates.

Results

The proteome comparison of T47D and MCF7 cells

To compare the proteomes of T47D and MCF7 cells, we performed 2-D gel analysis using total protein lysates of the two cell lines. The analysis was carried out on six 2-D electrophorisis gels made from three independent protein samples of each cell line. The Progenesis Discovery software package was used to carry out statistical comparative analyses of the proteomic profiles of T47D and MCF7 cells. The two cell lines displayed similar spot patterns, which allowed a good spot alignment (Figure 1A). T47D protein samples exhibited 298 supplementary spots compared to MCF7 (Figure 1B), suggesting that the former cells express a higher number of proteins than the latter. The proteomic analyses using the Progenesis software and a t-test (with a p-value<0.05) identified 97 significant differential spots as follows: 70 spots exhibited a variation≥2-fold, including 31 spots up-regulated and 39 spots down-regulated in T47D, whereas 12 and 15 spots were found unique to T47D and MCF7, respectively (Figure 1B). Some differentially expressed spots are shown in more detail in Figure 1A.

For the next step, MS identification, protein spots were selected among those uniquely and strongly (more than 3-fold difference) overexpressed, but also among those weakly (2 to 3-fold difference) up-regulated and well defined in each cell. The selection of weakly up-regulated spots was relevant to detect small proteomic differences between cells. A total of 40 spots were excised from Sypro Ruby-stained 2-D gels and were subjected to trypsin digestion. The resulting peptide fragments were analyzed by MS. Proteins with known Uniprot accession numbers were identified in all the 40 spots. The numbers of proteins revealed by MS analysis are listed in Figure 1B. A total of 205 proteins were identified from
### Table 2. Mass spectrometry identification of proteins in spots up-regulated in or unique to MCF7 as compared to T47D cell line.

| Spot | FC | Description | UniProt number | MW exp/ pred (kDa) | pI exp | Pep | Function description and/or biological process |
|------|----|-------------|----------------|--------------------|-------|-----|-----------------------------------------------|
| 2808 | 3.2 | Triosephosphate isomerase | P60174         | 28/27              | 6.2   | 19  | Fatty acid biosynthesis, glycolysis         |
|      |     | Protein ETH1, mitochondrial |               |                    |       |     |                                               |
| 2212 | 2.0 | Heterogeneous nuclear ribonucleoprotein H3 | P31942       | 42/37              | 6.5   | 13  | mRNA processing                             |
|      |     | Allotaxin B1 aldehyde reductase member 2 | O43488       | 42/40              | 6.5   | 8   | Cellular aldehyde metabolism               |
|      |     | Glycerol-3-phosphate dehydrogenase 1-like | Q8N335       | 42/38              | 6.5   | 6   | Glycerol-3-phosphate catabolism           |
|      |     | Heterogeneous nuclear ribonucleoprotein D-like | O14979      | 42/46              | 6.5   | 2   | Transcription regulation                   |
| 4384 | 5.2 | Latexin | Q9BS40         | 31/26              | 5.5   | 14  | Metalloendopeptidase inhibition            |
|      |     | Proteasome activator complex subunit 1 | Q06323       | 31/29              | 5.5   | 4   | Immunoproteasome assembly                  |
|      |     | cDNA, FLJ96310 | FLJ4267, highly similar to Homo sapiens glutathione S-transferase | B2R83     | 32/28 | 5.5 | Metabolism                                  |
|      |     | NAD-dependent deacetylase sirtuin-3, mitochondrial | Q9NTG7      | 32/44              | 6.2   | 2   | NAD-dependent deacetylase activity        |
| 3427 | 3.3 | SH3 domain-binding glutamic acid-rich-like | Q7S368       | 17/13              | 5.2   | 14  | SH3 domain binding, SH3/SH2 adaptor        |
| 3184 | 2.7 | Myosin regulatory light chain 2, nonsarcomeric | P19105       | 21/20              | 4.6   | 7   | Cytokinesis, receptor capping, cell locomotion |
| 3405 | 2.4 | Prolin | Q4V994        | 21/15              | 4.6   | 12  | Actin binding                               |
|      |     | Cellular retinoid acid-binding protein 1 | P29762       | 21/16              | 4.6   | 2   | Transport                                   |
|      |     | TRIM12-like protein | Q9U30 | 21/14              | 4.6   | 2   | Protein binding                            |
| 3102 | 2.2 | Chromobox protein homolog 3* (3102) | Q13188       | 21/21              | 5.1   | 11  | Transcription regulation                   |
|      |     | BH3-interacting domain death agonist p11 | P59957       | 21/22              | 5.1   | 2   | Apoptosis                                   |
|      |     | Chromobox protein homolog 5 | P45973       | 21/22              | 5.1   | 2   | Repressor of transcription, chromatin assembly or disassembly |
| 3095 | 7.0 | Cytochrome c-releasing factor 21* | O7S223       | 23/21              | 5.0   | 5   | Induction of apoptosis                      |
| 1968 | 4.7 | Interleukin-10 | P22301       | 50/21              | 5.0   | 2   | Inhibits the synthesis of cytokines, cell proliferation, anti-apoptosis |
|      |     | Telomerase-binding protein EST1A | Q86US8       | 50/160             | 5.0   | 2   | Telomere regulation, nonsense-mediated mRNA decay |
|      |     | Bullous pemphigoid antigen 1, isoforms 6/9/10 | O94833       | 50/591             | 5.0   | 2   | Cell adhesion                               |
|      |     | cAMP response element-binding protein | P16620       | 50/37              | 5.0   | 2   | Host-virus interaction, transcription       |
|      |     | Coiled-coil domain-containing protein 110 | Q878220      | 50/97              | 5.0   | 2   | Unknown                                    |
| 1870 | 5.7 | Transcriptional repressor NF-X1 | Q12986       | 53/124             | 5.1   | 2   | Transcription regulation                   |
|      |     | MHC class I antigen (Fragment) | A2TEM8       | 53/11              | 5.1   | 2   | Immune response                            |
|      |     | Probable global transcription activator SNF2L2 | B1AFLG3      | 53/181             | 5.1   | 2   | Transcriptional coactivator cooperating with nuclear hormone receptors |
| 4439 | U   | Thioredoxin-dependent peroxide reductase, mitochondrial| P30048       | 25/28              | 6.2   | 2   | Redox regulation of the cell               |
| 4492 | U   | Transgelin-2 | P37802       | 20/22              | 5.6   | 8   | Muscle development                         |
| 4404 | U   | Pyridoxine 5'-phosphate oxidase (F.) | Q53FP0       | 29/30              | 5.8   | 5   | Pyridoxine biosynthesis                     |
| 3563 | U   | Isopentenyl-diphosphate Delta-isomerase 1 | Q13907       | 29/26              | 5.8   | 4   | Steroid, lipid, carotenoid biosynthesis    |
| 4572 | U   | Protein S100-A14 | Q9HCY8       | 16/12              | 5.0   | 4   | Calcium ion binding                        |
| 4530 | U   | Calmodulin-like 5 | Q550Q3       | 18/16              | 4.2   | 4   | Calcium ion binding                        |
| 4094 | U   | NADP-dependent malic enzyme | P48163       | 75/64              | 5.8   | 24  | Nadp biosynthetic process                  |
|      |     | T-complex protein 1 subunit alpha | P17987       | 75/60              | 5.8   | 12  | Protein folding                            |
|      |     | Prolyl 4-hydroxylase subunit alpha-1 | P13674       | 75/61              | 5.8   | 10  | Protein metabolic process                  |
|      |     | Heterogeneous nuclear ribonucleoprotein K | P61978      | 75/51              | 5.8   | 5   | Host-virus interaction, mRNA processing    |
|      |     | Beta-galactosidase | A8MZ11       | 75/73              | 5.8   | 3   | Glyceridase, hydrolase                     |
|      |     | REST corepressor 1 | Q9UKL0       | 75/53              | 5.8   | 2   | Transcription regulation                   |
these 40 spots, with a number of them found in multiple spots. For example, nitrilase homolog 2 was found in only one spot, the spot number 4364, while cathepsin D was found in several spots including spot numbers 2685 (Figure 1A). Consequently, distinct proteins amount to 164 with 52 and 16 proteins from spots unique to T47D and MCF7, respectively, and 96 proteins from

| Spot | FC | Description                  | UniProt number | MW exp/pred (kDa) | pl exp | Pep | Function description and/or biological process                                                                 |
|------|----|------------------------------|----------------|-------------------|-------|-----|-------------------------------------------------------------------------------------------------|
| 4364 | U  | Nitrilase homolog 2*         | Q9NQR4         | 32/31             | 5.9   | 4   | Decreases the colony-forming capacity of cultured cells by arresting cells in the G2 phase of the cell cycle |

The function description and/or biological process and the subcellular location were from the Uniprot database (www.uniprot.org). Spot, spot number; FC, fold change; MW, molecular weight; pl exp, isoelectric point as determined from the 2-D gel experiments; Pep, number of unique peptides; U, unique. The number after the protein name indicated the additional spot in which the protein was found.

*Protein previously reported to be used as a breast cancer biomarker and which were overexpressed in cancerous cells based on data from the literature [9,10].

Protein used for Q-RT-PCR validation.

doi:10.1371/journal.pone.0031532.t002

**Figure 2. Functions and cellular locations of the differentially expressed proteins in T47D and MCF7 cells identified by the proteomics approach.** The Uniprot database was used to generate the cellular location and the molecular function and/or biological process of each of the 164 non-redundant (distinct) proteins identified by mass spectrometry analysis as differentially regulated in T47D as compared to MCF7 cells.

doi:10.1371/journal.pone.0031532.g002
differential spots. These results revealed that T47D and MCF7 cells present some significant differences in regard to their proteomes.

### Functional and subcellular protein categorizations

Using the UniProt database at www.uniprot.org, we determined the functions and/or biological processes of each identified protein (Table 1, 2 and S1). Table 1 and Table S1 list the proteins found in spots up-regulated or unique to T47D as compared to the MCF7 cell line while Table 2 lists the proteins found in spots down-regulated in T47D or unique to MCF7. The spot from which each protein was identified, the spot fold-increase or fold-decrease in one cell line versus the other cell line, the protein name, the molecular weight, the isoelectric point, the number of unique peptides allowing the identification of each protein in the MS analysis, and the UniProt accession number of the protein, were mentioned. The information about the molecular function and/or biological process and subcellular location was found for most proteins.

The reparation of each function and subcellular location are illustrated in Figure 2. From the 164 proteins identified by MS, 14 were principally implicated in transport, 13 in metabolism, 11 in apoptosis, 9 in proteolysis, 8 in transcription, 7 in mRNA processing and 7 in RNA and protein binding. Differentially expressed proteins are mainly located in cytoplasm and nucleus.

The proteomic comparisons notably led to the identification of five proteins that are used as breast cancer diagnostic and prognostic biomarkers: proliferating cell nuclear antigen (PCNA), cathepsin B, heat shock protein beta-1 (HSP27) [9,10,14,15] (Table 1 and 2), PCNA was identified in a spot 6.1 times up-regulated in T47D, as compared to MCF7. Cathepsin D was found in eight different spots, whereas cathepsin B was found in a unique spot up-regulated in T47D as compared to MCF7, and protein S100-A14, in a spot unique to MCF7 as compared to T47D. HSP27 was found in four different spots: two are overexpressed in T47D and two are overexpressed in MCF7. These results showed that breast cancer biomarkers are differentially expressed in the two breast cancer cell lines.

### Comparison of protein and transcript expression

Next, we investigated the mRNA expression of proteins identified in the proteomic analyses to evaluate if there is a correlation between protein and mRNA expression. To do this, eight proteins principally involved in steroid metabolism, cell proliferation and apoptosis were selected: 17β-HSD type 10, PCNA, cathepsin B, nitrilase homolog 2, CDV3 homolog, heat shock 70 kDa protein 1, cytochrome c-releasing factor 21. Their mRNA levels were quantified by quantitative real-time RT-PCR (Q-RT-PCR) analysis of total RNA extracts from the two cell lines T47D and MCF7. Proteomics and Q-RT-PCR data were considered to correlate if the mRNA level and protein spot were regulated in the same direction (Table 3). Except for one protein, all the other seven proteins for which the mRNA expression was evaluated, exhibited a regulation in the same direction at protein and mRNA levels in T47D as compared to MCF7. These data can indicate the existence of a semiquantitative correlation between protein and mRNA expression. Thus, it may be possible to predict the presence of a protein based on its gene expression and inversely.

In parallel work, we also measured mRNA levels of various proteins involved in estradiol (E2) synthesis, inactivation and

| Table 3. Q-RT-PCR values (thousand copies of mRNA/μg total RNA) of mRNAs encoding various enzymes involved in estradiol production (or action) and breast cancer cell proliferation within T47D and MCF7 and comparison with 2-D gel data. |
|---------------------------------|----------------|----------------|-----------------|
| **Description**                | **T47D**       | **MCF7**       | **Correlation 2-D gel and Q-RT-PCR** |
| Proliferating cell nuclear antigen (PCNA) | 2,333          | 1,600          | Yes             |
| Protein CDV3 homolog           | 592            | 419            | Yes             |
| 17β-HSD type 10                | 1,740          | 1,477          | Yes             |
| Cathepsin B                    | 1,425          | 1,255          | Yes             |
| Heat shock 70 kDa protein 1 (HSP70.1) | 725            | 1,926          | No              |
| Chromobox protein homolog 3    | 1,321          | 1,966          | Yes             |
| Cytochrome c-releasing factor 21| 19             | 29             | Yes             |
| Nitrilase homolog 2            | 435            | 791            | Yes             |
| 17β-HSD type 1                 | 697            | 83             | nd              |
| 17β-HSD type 2                 | N              | N              | nd              |
| 17β-HSD type 5                 | 4              | 850            | nd              |
| 17β-HSD type 7                 | 150            | 159            | nd              |
| 17β-HSD type 12                | 362            | 101            | nd              |
| Aromatase (P450arom)           | 0              | N              | nd              |
| Estrogen sulfotransferase (EST) | 0              | 0              | nd              |
| Steroid sulfatase (STS)        | 60             | 80             | nd              |
| Estrogen receptor alpha (ERα)  | 190            | 653            | nd              |
| Estrogen receptor beta (ERβ)   | 8              | 7              | nd              |
| Androgen receptor (AR)         | 127            | 96             | nd              |

N, negligible (Q-RT-PCR values <1 thousand copies of mRNA/μg total RNA); 0, mRNA not detected after many rounds of amplification; nd, not determined. SDs were <10% of duplicates.

Proteins in bold were selected for Q-RT-PCR validation after their identification by mass spectrometry analysis of 2-D gel spots.

Peter J. Robison et al. (2012) *Proteomic Analyses of Breast Cancer Cell Lines* PLoS ONE 7(2), e31532. doi:10.1371/journal.pone.0031532.t003
action in the two cell lines for comparison. These proteins include several 17β-hydroxysteroid dehydrogenase (17β-HSD) enzymes and steroid receptors (Table 3). Results showed that mRNAs of 17β-HSD types 1 and 12 and androgen receptor (AR) are expressed in greater amounts in T47D than in MCF7, whereas mRNAs of 17β-HSD type 5, estrogen receptor alpha (ERα) and steroid sulfatase are less expressed. The major difference however concerned the transcript of 17β-HSD type 5 which was 189 times lower in T47D than in MCF7. 17β-HSD type 7 was expressed at about the same level in both cell lines while mRNA levels of 17β-HSD type 2, aromatase and estrogen sulfotransferase were negligible or near zero in both cell lines (Table 3). These data show that the transcripts of enzymes and steroid receptors involved in E2 production and action are differentially expressed in the two cell lines. The correlation between protein and mRNA expression was not determined for these enzymes since they were not identified in the proteomic analyses.

Discussion

T47D and MCF7 cells are two ER positive hormone-dependent breast cancer cell lines which additionally express AR. The two cell lines are widely used for the studies of breast cancer mechanisms. Proteomic studies of individual cell lines have been previously reported [8–10] but the present study established the first protein profile comparison between the two cell lines. The overlaid 2-D gel images of T47D and MCF7 showed similar spot patterns, reflecting their common origin, pleural effusion from mammary gland tumor metastasis (ATCC, www.atcc.org). Proteomic data suggest that T47D expresses a higher number of proteins than MCF7; in agreement with the understanding of cell evolution, this indicated that the first cell line could exhibit a higher number of functional mRNAs and/or more active proteins than the latter cell line.

MS analyses indicated that 18 proteins (Table 1, 2 and S1) were present in more than one spot on the gel. These proteins included heat shock protein beta-1 (implicated in stress resistance), prohibitin (implicated in cell proliferation), chromobobrox protein homolog 3 (implicated in transcription regulation) and cathepsin D (implicated in proteolysis). For example, cathepsin D was found in eight spots of which five were up-regulated and three were down-regulated in T47D. The presence of a protein in several spots and its regulation in different directions among the spots can be due to post-translational modifications like phosphorylation, glycosylation, or limited proteolytic cleavage [8]. The up-regulated cathepsin D in T47D exhibited an apparent molecular weight (MW) of about 28 kDa (25, 28 and 29 kDa), whereas the down-regulated comprised a protein exhibiting a MW of 31–32 and 50 kDa. The presence of cathepsin D with several apparent MW reflects the existence of isoforms. In fact, human cathepsin D is synthesized as pre-pro-enzyme that undergoes a co-translational removal of peptide and several proteolytic cleavages to generate the pro-enzyme (a 52 kDa pro-cathepsin D), an enzymatically active 44–48 kDa intermediate and a two-chain form with non-covalently associated subunits of 14 and 31–34 kDa [8,16,17]. The up-regulation of the 28 kDa isoform and the down-regulation of the 50 kDa protein in T47D cells may indicate that the pro-cathepsin D is down-regulated in this cell line. This may explain why a cathepsin D with a MW higher than 33 kDa was not identified in a previous T47D and antiestrogen-resistant derivative T47D-r study [8].

Several proteins involved in cell growth and anti-apoptosis regulations and cancerogenesis are more strongly expressed in T47D than in MCF7. These proteins include caspase-3 subunit p12, Nuclear protein Hcc-1, G1/S-specific cyclin-D3, cathepsin B, protein CDV3 homolog, N(G),N(G)-dimethylarginine dimethylaminohydrolase 2 and prohibitin. Other proteins implicated in transcription repression and apoptosis regulation are, on the contrary, less abundantly expressed in T47D than in MCF7. These are chromobox protein homologs 3 and 5, BH3-interacting domain death agonist p11, cytochrome c-releasing factor 21, transcriptional repressor NF-X1, nitrilase homolog 2 and interleukin-10. From these data, it appears that proteins implicated in cell proliferation stimulation seem to be more up-regulated in T47D as compared to MCF7, whereas proteins involved in cell growth repression are therein down-regulated. Our study showed that proteins that are differentially expressed between T47D and MCF7 are implicated in all the biological functions of the cell. An example is ‘DNA replication’ which contributes to cell proliferation. In addition, markers classified as risk markers (genetic), prognostic factors (which correlate with patient outcome), predictive markers (prediction of the response or resistance to a specific therapy) and markers for the follow-up of patients with diagnosed cancer (recurrent disease detection or treatment monitoring) [9,10,14,15] are found to be differentially expressed in the two cell lines. Most of these markers have been tested on tumor samples, and they are overexpressed or mutated in a significant proportion of breast cancers. The best-known molecular risk marker is PCNA [14].

Estrogens are clearly carcinogenic in humans but the molecular pathways by which these hormones induce cancer are only partially understood [18]. To improve our understanding of these pathways, the mRNA expression of enzymes implicated in E2 synthesis and action were evaluated in T47D and MCF7 cell lines. Our study shows that aromatase is not expressed in T47D and its expression in MCF7 is negligible compared to those of the 17β-HSD enzymes, indicating that E2 synthesis in these two cell lines proceeds mainly by 17β-HSD activities certainly via the steroid sulfatase pathway. Our results also permit a comparison of the relative mRNA expression levels of the three sex-hormone receptors, ERα, ERβ and AR, in T47D and MCF7 cells. In both cell lines, AR and ERα are more highly expressed than ERβ with ERα having the highest expression. In MCF7, AR, the cognate receptor of dihydrotestosterone (DHT), an androgen that decreases the estradiol-dependent growth of breast cancer cells [7,19,20], was 6.8 times less expressed than ERα, whereas the difference is only 1.5 times in T47D. This suggests that AR-mediated activities are higher in the latter cell line than in the former. DHT may thus contribute to the decrease of E2-dependent growth more efficiently in T47D than in MCF7 cells.

In conclusion, the present study reveals that a high number (at least 164) of proteins (including proteins involved in breast cancer cell growth regulation) are differentially expressed between the two most used human breast cancer cell lines, T47D and MCF7. This suggests that these proteins, listed in Tables 1, 2 and S1, could be differentially expressed in breast tumors. The list of differentially expressed proteins generated in the present study may provide useful information for further studies of breast cancer mechanisms with T47D and MCF7, as breast cancer cell models.

Supporting Information

Table S1 Additional data of mass spectrometry identification of proteins in spots up-regulated in or unique to T47D as compared to MCF7 cell line. The function description and/or biological process were from the UniProt database (www.uniprot.org). Spot, spot number; FC, fold change;
MW, molecular weight; pI exp, isoelectric point as determined from the 2-D gel experiments; Pep, number of unique peptides; U, unique. The number after the protein name indicated the additional spot in which the protein was found.

Acknowledgments

We thank Mrs G. Racine for her advice on the 2-D gel image analysis; we acknowledge Dr. E-L. Calvo for his critical reading of the manuscript, from the CHUQ Research Center (CHUL pavilion, Québec, Canada).

Author Contributions

Conceived and designed the experiments: JAA SXL. Performed the experiments: JAA. Analyzed the data: JAA. Contributed reagents/materials/analysis tools: JAA. Wrote the paper: JAA SXL.

References

1. Sasco AJ (2003) Breast Cancer and the Environment. Horm Res 60 Suppl 3: 50.
2. ESHRE Capri Workshop Group (2004) Hormones and breast cancer. Hum Reprod Update 10: 281–293.
3. Jemal A, Siegel R, Ward E, Hao Y, Xu J, et al. (2008) Cancer statistics, 2008. CA Cancer J Clin 58: 71–96.
4. Canadian Cancer Society’s Steering Committee (2009) Canadian Cancer Statistics 2009. Toronto, Canada, Canadian Cancer Society.
5. Husen B, Huhitinen K, Saloniemi T, Messinger J, Thele, et al. (2006) Human hydroxysteroid (17-beta) dehydrogenase 1 expression enhances estrogen sensitivity of MCF-7 breast cancer cell xenografts. Endocrinology 147: 5333–5339.
6. Day JM, Foster PA, Tuill HJ, Parsons MF, Neuman SP, et al. (2008) 17β-hydroxysteroid dehydrogenase Type 1, and not Type 12, is a target for endocrine therapy of hormone-dependent breast cancer. Int J Cancer 122: 1931–1940.
7. Aka JA, Mazumdar M, Chen CQ, Pozier D, Lin SN (2010) 17β-hydroxysteroid dehydrogenase Type 1 stimulates breast cancer by dihydrotestosterone inactivation in addition to estradiol production. Mol Endocrinol 24: 832–845.
8. Huber M, Badr I, Kratzschmar JR, Becker A, Muller EC, et al. (2004) Comparison of Proteomic and Genomic Analyses of the Human Breast Cancer Cell Line T47D and the Antiestrogen-resistant Derivative T47D-r. Mol Cell Proteomics 3: 43–55.
9. Bianchi L, Canton C, Bisi L, Orlandi R, Meinard S, et al. (2003) Protein profile changes in the human breast cancer cell line MCF-7 in response to SEL1L gene induction. Proteomics 5: 2433–2442.
10. Sarvari HA, Yoon JH, Lazar IM (2006) Proteome profile of the MCF7 cancer cell line: a mass spectrometric evaluation. Rapid Commun Mass Spectrom 20: 3039–3055.
11. Shevchenko A, Wilm M, Vorm O, Mann M (1996) Mass spectrometric sequencing of proteins from silver-stained polyacrylamide gels. Analytical Chemistry 68: 650–658.
12. Hueh J, Thomas H, Schela M, Shevchenko A (2003) Fast-Response Proteomics by Accelerated In-Gel Digestion of Proteins. Anal Chem 75: 1300–1306.
13. Lau-Thé V, Paquet N, Calvo E, Cumps J (2005) Improved real-time RT-PCR method for high-throughput measurements using second derivative calculation and double correction. Biotechniques 39: 297–293.
14. Journet A, Ferro M (2004) The potentials of MS-based subproteomic approaches in medical science: the case of lysosomes and breast cancer. Mass Spectrom Rev 23: 393–442.
15. Gast MC, Schellen JJ, Beijnen JH (2008) Clinical proteomics in breast cancer: a review. Breast Cancer Res Treat 116: 17–29.
16. Faust PL, Wall DA, Perera E, Lingappa VR, Kornfeld S (1987) Expression of human cathepsin D in Xenopus oocytes: phosphorylation and intracellular targeting. J Cell Biol 105: 1937–1945.
17. Laude-J-Coopman E, Beaujouin M, Derocq D, Garcia M, Glondu-Lassis M, et al. (2006) Cathepsin D: newly discovered functions of a long-standing aspartic protease in cancer and apoptosis. Cancer Lett 237: 167–179.
18. Parli FF (2000) Estrogen, estrogen receptor and breast cancer. Biomedical and Health Research Vol 36. Amsterdam: IOS Press.
19. Ashinall SR, Stamp S, Davison A, Shannon BK, Leonard TW (2004) The proliferative effects of 5-androstene-3 beta,17 beta-diol and 5 alpha-dihydrotestosterone on cell cycle analysis and cell proliferation in MCF7, T47D and MDAMB231 breast cancer cell lines. J Steroid Biochem Mol Biol 83: 37–51.
20. Greeve MA, Allan RK, Harvey JM, Beaton JM (2004) Inhibition of MCF-7 breast cancer cell proliferation by 5alpha-dihydrotestosterone, a role for p21(Cip1/Waf1). J Mol Endocrinol 32: 789–810.