Microarray Analysis of Breast Cancer Gene Expression Profiling in Response to 2-Deoxyglucose, Metformin, and Glucose Starvation

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Abstract

Background: Breast cancer (BC) is the most frequently diagnosed cancer in women. Altering glucose metabolism and its effects on cancer progression and treatment resistance is an emerging interest in BC research. For instance, combining chemotherapy with glucose-lowering drugs (2-deoxyglucose (2-DG), metformin) or glucose starvation (GS) has shown better outcomes than with chemotherapy alone. However, the genes and molecular mechanisms that govern the action of these glucose deprivation conditions have not been fully elucidated. Here, we compared the effect of glucose deprivation (2-DG, metformin, GS) on MDA-MB-231 and MCF-7 BC cell lines using microarray analysis to establish a database of differentially expressed genes and enrichment pathways, in order to investigate the beneficial effect of glucose-lowering treatments on the vulnerability of BC cells.

Methods: MDA-MB-231 and MCF-7 cells were treated with 20 mM metformin or 4 mM 2-DG for 48 hours. GS was performed by gradually decreasing the concentration of glucose in the culture medium to 0 g/L with fetal bovine serum over 1 week. Expression profiling was carried out using Affymetrix Human Clariom S microarrays. Differentially expressed genes were obtained from the Transcriptome Analysis Console and enriched using DAVID and R packages.

Results: Our results showed that MDA-MB-231 cells were more responsive to glucose deprivation than MCF-7 cells. Endoplasmic reticulum stress response and cell cycle inhibition were detected after all three glucose deprivations in MDA-MB-231 cells and only under the metformin and GS conditions in MCF-7 cells. Induction of apoptosis and inhibition of DNA replication were observed with all three treatments in MDA-MB-231 cells and metformin-treated MCF-7 cells. Upregulation of cellular response to reactive oxygen species and inhibition of DNA repair mechanisms resulted after metformin and GS administration in MDA-MB-231 cell lines and metformin-treated MCF-7 cells. Autophagy was induced after 2-DG treatment in MDA-MB-231 cells and after metformin in MCF-7 cells. Finally, enhanced cell-cell adhesion and inhibition of DNA methylation and cholesterol biosynthesis were observed with GS only in MDA-MB-231 cells.

Conclusion: GS had the greatest effect on breast cancer cells compared to 2-DG and metformin. Our results suggest that the combination of metformin and GS should weaken both cell lines and makes them more vulnerable to conventional chemotherapy.

Background

Breast cancer (BC) is the most common female malignancy and the second leading cause of cancer-related death in women [1], with more than 500,000 deaths worldwide each year [2]. Based on molecular marker expression, BC is divided into subtypes as follows: hormone receptor (HR)-positive subtype (estrogen (ER+) or progesterone receptor (PR+)), human epidermal growth factor receptor 2 overexpression subtype (HER2 +), and triple-negative breast cancer (TNBC) (ER-, PR-, HER2-) [3]. The subtypes of TNBC present as basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR) [4]. This classification into subtypes is useful when selecting the most appropriate therapy.

For patients with HR-positive BC, endocrine therapy is the preferred treatment approach. The most common adjuvants are the estrogen modulator Tamoxifen and the aromatase inhibitors (letrozole, anastrozole, and exemestane), while chemotherapy is reserved for patients at risk of visceral crisis or with endocrine resistance [5]. For patients with HER2-positive BC, the combination of an anti-HER2 monoclonal antibody (Trastuzumab, Pertuzumab) with chemotherapy is the standard treatment [6]. Compared with other types of breast cancer, TNBC is the most difficult subtype to treat because it lacks appropriate targets for molecular therapy; therefore, chemotherapy remains the main approach to first-line treatment of TNBC [7]. However, some cases of BC develop resistance to chemotherapies or fail to respond to conventional therapies due to altered tumor glucose metabolism [8]. This aberrant metabolism is characterized by hyperactivated aerobic glycolysis, a phenomenon coined “Warburg effect” [9], and decreased oxidative phosphorylation (OXPHOS). Controlling these phenomena using metabolic inhibitors such as the antidiabetic agent metformin (MET) and 2-deoxyglucose (2-DG), or by inducing glucose starvation (GS) should therefore be a promising approach to overcome therapy resistance [10].

The combination of chemotherapeutic drugs and MET is being clinically tested for efficacy in the treatment of cancer. The credibility of MET use is related to MET's ability to target cancer cell metabolism and interrupt tumor progression [11]. MET-mediated inhibition of mitochondrial respiratory chain complex 1 increases levels of adenosine monophosphate (AMP), which activates AMP-activated protein kinase (AMPK). The latter activation in turn inhibits the mammalian target of Rapamycin (mTOR), which is responsible for cell proliferation [12]. In addition, AMPK activation suppresses many metabolic processes (gluconeogenesis, protein and fat synthesis, glycolysis, and fatty acid beta-oxidation) [13]. It also induces cell cycle arrest and apoptosis through down-regulation of the tumor protein p53 (p53) and exerts anti-inflammatory actions by inhibiting nuclear factor kappa B (NF- κ B) pathways [14].

The anticancer agent 2-DG is also considered to target the metabolic homeostasis of a cell. Clinical trials have suggested that combining 2-DG with chemotherapies overcomes drug resistance by eliminating breast cancer stem cells (BCSC)-induced resistance [15]. This glucose decoy is phosphorylated by hexokinase 2 (HK2) to inhibit glycolysis, resulting in adenosine triphosphate (ATP) depletion and inducing BC cell death [16]. 2-DG can alter the redox state of a cell, leading to apoptosis. It can also contribute to the inhibition of protein glycosylation, leading to endoplasmic reticulum (ER) stress and activation of the unfolded protein response (UPR) [17].

Glucose addiction in cancer is linked to hyperproliferation [18]. Inhibition of glycolytic activity via GS could be a potential therapeutic approach [19]. Clinical studies have shown a correlation between caloric intake and breast cancer etiology. In addition, a lower incidence of cancer and a longer life span in men are observed after a low-sugar diet. GS inhibits energy-dependent signaling pathways, including the insulin-like growth factor-1/phosphoinositide 3-kinase/protein kinase B/mTOR (IGF-1/PI3K/Akt/mTOR) pathway, and activates AMPK resulting in down-regulation of glycolysis activity and cell proliferation [20]. In addition, GS increases ketone bodies in the bloodstream, which protects mitochondria from inflammation and reactive oxygen species (ROS) during cancer treatment [21]. It also decreases lactate production to prevent intracellular alkalinization [22].

In this study, our objective was to identify differentially expressed genes (DEGs) in estrogen and progesterone receptor-positive (MCF-7) and triple-negative (MDA-MB-231) BC cell lines treated with different glucose deprivation (metformin, 2-DG, or GS) using microarray analysis. DEGs were enriched using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) and R packages to highlight differentially enriched genes and pathways. Our study aims to investigate the beneficial effect of glucose-lowering on the vulnerability of BC cells.

Methods

Reagents

2-Deoxy-D-glucose (2-DG), 1,1-dimethyl biguanide hydrochloride (Metformin), penicillin/ streptomycin solution (100x), fetal bovine serum (FBS), trypsin solution, Dulbecco's modified Eagle's medium (DMEM) (Glucose-free, L-glutamine, phenol red, sodium pyruvate and sodium bicarbonate powder suitable for cell culture), Dulbecco's modified Eagle's medium high glucose (DMEM, 4500 mg/L), Dulbecco's modified Eagle's medium low glucose (DMEM, 1000 mg/L) were bought and imported from Sigma-Aldrich (St. Louis, MO, USA). NucleoZol was purchased from MACHEREY-NAGEL (Bethlehem, PA, USA). GeneChip™ WT PLUS reagent kit was obtained from Thermo Fisher Scientific (Waltham, MA, USA).

Cell culture

BC cell lines MDA-MB-231 (ATCC®HTB-26™) and MCF-7 (ATCC®HTB-22™) were purchased from the American type culture collection (ATCC, Manassas, Virginia, USA). Cells were grown in DMEM 4500 mg/L glucose supplemented with 10% FBS and 1% penicillin/streptomycin (100x) and treated with 20 mM MET or 4 mM 2-DG for 48 hours. GS was executed by gradually decreasing the concentration of glucose in the culture medium from 4.5, to 1, to 0.5 g/L, until a concentration of 0 g/L was reached. The cells remained in this zero-glucose medium for 1 week, with 10% FBS and 1% penicillin/streptomycin 100x. The culture medium was changed daily. Cells were maintained at 37 °C in a humidified atmosphere, with 5% CO₂.

RNA extraction and gene expression microarrays

Total RNA from 3 independent experiments (biological replicates) was extracted using NucleoZol according to the manufacturer's instructions. RNA concentration and A260/A280 ratio were determined using the NanoDrop™ 1000 spectrophotometer (Thermo Scientific). RNA integrity was assessed by denaturing agarose gel electrophoresis (1%). One hundred ng of total RNA was reverse transcribed following the instructions of the GeneChip® WT Plus Reagent Kit (Affymetrix, Inc., Santa Clara, CA, USA). Complementary RNA (cRNA) was synthesized and amplified through *in vitro* transcription (IVT) using T7 RNA polymerase (using the Affymetrix WT cDNA Synthesis and Amplification Kit). The transcription was done having second-stranded cDNA as a template. After purification, the sense strand cDNA was synthesized by reverse transcription of the cRNA using 2nd cycle primers. The cRNA template was then hydrolyzed using RNase H., and 5.5 µg of ss-cDNA purified, fragmented, and labeled with biotin according to the Affymetrix WT End Labeling Kit. The ss-cDNA was then hybridized to the Clariom™ S human transcriptome array (Affymetrix, Inc., Santa Clara, CA, USA) at 45 °C for 17 h using the GeneChip™ 645 hybridization oven. The arrays were washed, stained on the FS450 Fluid Station, and scanned using the GeneChip Scanner 3000 7G (Affymetrix, Inc., Santa Clara, CA, USA) according to the GeneChip™ User Guide. The raw CEL files containing the intensity data were extracted analyzed as described in the following.

Statistical and Bioinformatics analysis

Analysis protocols. Two microarray data mining protocols were adopted. The gene expression data were extracted in each protocol and CEL files were generated using the Thermo Fisher Transcriptome Analysis Console (TAC) with the SST-RMA as the normalization algorithm [23]. Each strategy has its limitations but combined they added valuable data checks to the final results.

Gene sets. Both protocols start by comparing, in TAC, three sets of treatment probes to three sets of control probes in each cancer cell line separately. Genes with a "Fold Change" (FC) <-2 or > 2 and having a p-value <0.05 were regarded as significantly differentially expressed. The interrogated genes were then divided into two groups: those that are upregulated (i.e., FC> 2) and those that are downregulated (i.e., FC<-2). The "Volcano plots" for each comparison were exported simultaneously. In addition, the overlapping genes between the two cancer types in each of the upregulated and downregulated treatments, and the set merging the latter two, were determined using "BioinfoGP Venny" [24]. This latter set will be referred to as the "overlap" set. The intersection of the sets of up-regulated and then down-regulated genes from the three treatments in each cell line, including the overlap, was also performed using Venny. Then, each subsection of the intersected Venn diagram was merged with its other pair. Another final set was queried from the comparison of controls in each of the two lines, still using the same FC criteria. A simplified schematic of the DEGs' partitioning is shown in Fig. 1.

First Protocol. The first protocol proceeded by composing the gene sets in the Database for Annotation, Visualization, and Integrated Discovery (DAVID) for gene enrichment analysis and clustering [25]. Selected enrichment terms included the gene ontology (GO) GOTERM_BP_DIRECT, GOTERM_CC_DIRECT, and GOTERM_MF_DIRECT and the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome for pathway enrichment. The DAVID functional annotation clustering tool was used, and clusters were exported to Excel files for further analysis. A significant number of non-clustered terms were relatable to the clustered terms. The latter had a p-value <0.05 and a Benjamini p-value rapidly becoming insignificant with lower cluster "Enrichment Scores". To account for the precedent, a geometric mean <0.2 of the p-value of all terms of a cluster was considered sufficient to retain this cluster. On the other hand, if no significant cluster was found, the non-clustered terms with a Benjamini p-value <0.05 were retained, and/or the analysis was checked with the results of the second method.

Second protocol. For the second protocol, "R" was used [26]. Comparison of treatments to controls within each cancer cell line, and between cell controls, was performed using significance analysis of microarray (SAM) [27]. The data type was set to "two-class unpaired" and the FC option "log scale" was checked. The "fitness factor Δ" was retained when it corresponded to a 90th percentile FDR <0.005, a median FDR as small as possible, and "called genes" as high as possible (>100 genes). The number of permutations was adjusted from a default value of 100 to 5000 when the cited requirements were not met. As a result,

the Δ and overall FDR values varied faintly around 0.5 and 0.002, respectively, and all gene FCs happened to be either <-2 or >2 . The results were then exported with their "SAM plots" and the up-and down-regulated sets were extracted. Intersections were performed using packages that are part of R, "gplots" and "ggVennDiagram" [28,29], and the same sets were drawn, including those of the "overlap" cell line. GO and Reactome pathway enrichment analyses were then performed on the sets, and terms with a Benjamini p-value <0.05 were retained only. The R packages "AnnotationDbi", "clusterProfiler" and "ReactomePA" were required for gene enrichment [30–32]. The terms were then converted into three semantic similarity matrices and 1 gene overlap similarity matrix, namely three for GOs and one for Reactome respectively. These matrices were subjected to "binary cut" clustering using "simplifyEnrichment" [33], as recommended by the software authors. The results were ordered and exported for analysis. It should also be noted that binary cut is not recommended for KEGG terms, which were covered by the first method.

Cluster analysis. In each protocol, clusters in the merged set (i.e., containing both up-and down-regulated genes) were labeled "up-/down-regulated" based on their cluster matches in the analyzed up-or down-regulated sets. It should be noted that the analysis of the subsets resulting from the intersection of the three treatments was performed using only BP GO and Reactome, and only common genes between treatments were analyzed. The previous decisions were made to avoid redundancy. Finally, the results from both methods were unified and presented as-is in this paper. GO BP heat maps were constructed from the data provided by the second method using "simplifyEnrichment" with binary clustering as the default method; in particular, the large sets containing all differentially expressed genes were mapped. Histograms showing DEGs were constructed using "cowplot," "ggplot2," and "dplyr" [34–36]. Apple's "Pages" software was useful for representing intersections in the Venn diagrams, which are not to scale.

Conserved genes. In addition, a nonparametric Kolmogorov-Smirnov test was used to infer genes that showed no change in expression after treatment. For each gene, two cumulative Student's standard error distributions were calculated from the two triplets of probes, i.e., control and treatment. These were then added to their respective expression means to give the cumulative probability distribution of the two means. The latter distributions were subtracted from each other to calculate the KS statistic. Genes with a statistic <0.2 were considered "conserved" and retained for enrichment analysis.

Results

The analysis was performed on two BC cell lines, MDA-MB-231 and MCF-7. DEGs were identified between the control of the two cell lines, and between treated cells, i.e., 2-DG, MET, or glucose-starved cells. DEGs were determined using TAC or SAM. DEGs were visualized either on a volcano or SAM plot (Fig. 2 a, b) (only the representation of MDA-MB-231-GS versus the MDA-MB-231 control is shown). The lists of DEGs and the others volcano and SAM plot representations are shown in Additional file 1: Table S1. Biological significance was extracted from the gene lists by systematically enriching their DEGs with GO and pathway terms (KEGG and Reactome for DAVID, only Reactome for binary clustering). The top enriched clusters are only described in this section. Modulators for the most significant terms are also shown in parentheses.

One should mind the language we used to describe the regulation of functions. This is recommended because the regulation of other regulations is highlighted. Regulation of regulation should be interpreted as if we were multiplying positive and negative numbers, e.g. if a cluster exhibits down-regulation, and if a function in that group negatively regulates a property A, the resulting regulation of A should tend towards a positive regulation. It should be noted that biological functions fall into two distinct categories: the modulation of a process and the process itself. Thus, for phrases such as "[negative regulation of] A", process A is under activity, coexisting with another process negatively modulating A. Furthermore, during the analysis of each cluster, terms describing positive and negative regulation coexisted. The regulation with a BH-adjusted p-value that was at least twenty times (i.e., $1:0.05$) greater than the other was assumed to be the dominant modulation. If this FC was less than twenty or if the p-value was >0.005 , the modulations were interpreted as canceling each other out.

The comparison between the cell line's controls can be reviewed with Additional file 2 and Additional file 3: Figure S1.

1. MDA-MB-231 cell line

1.1. Regulation with 2-DG

For the MDA-MB-231 cell line, the comparison of 2-DG-treated cells with the control (Fig. 3a) revealed that most clusters are up-regulated and very few are down-regulated. For the upregulated biological processes: [negative regulation of] response to stress and stimulus (lipids, hormones, chemicals), radiation, ER stress, and UPR (chaperone activation); [positive regulation of] cellular component traffic (protein and nitrogen compound localization, vesicle-mediated), cell migration; [negative regulation of] immune system processes (antigen presentation, toll-like receptors (TLRs), and interleukin (IL) signaling pathway); [positive regulation of] cell-cell and cell-substrate adhesion (laminin and proteoglycans); autophagy; [negative regulation of] metabolic processes of proteins and nitrogen compounds (phosphorylation, proteolysis); [negative regulation of] protein catabolism by ubiquitination; apoptotic processes; [positive regulation of] cell population proliferation (epithelial cells); [negative regulation of] homeostatic processes (temperature); and [positive regulation of] organization of extracellular components (collagen and hemidesmosome). These processes were found to recruit molecular functions describing protein binding, enzymes (kinase, ubiquitin ligase), adhesion molecules (cadherin), and glutamate receptors. In addition, oxidoreductase, protein disulfide isomerase, mitogen-activated protein kinase (MAPK), and transmembrane transporter activities were observed. Cellular compartments included cytoplasmic vesicles and lysosomes, nucleus and ER network, ER lumen and chaperone complex, cell adhesion foci, and the extracellular region.

Down-regulated clusters show DNA replication, DNA completion, cell cycle (G1/S phase transition), and purine/pyrimidine metabolism.

1.2. Regulation with metformin

Also comparing MDA-MB-231 cells, DEGs in MET-treated cells versus control (Fig. 3b) yielded mostly up-regulated clusters with a few down-regulated clusters. For the up-regulated clusters, the biological processes were [negative regulation of] RNA biosynthesis, phosphate metabolic process, and protein phosphorylation; [down-regulation of] stress and stimulus response (hypoxia, lipids, ROS, UPR, hormones, radiation, chemicals) and associated signal

transduction (cyclic adenosine monophosphate (cAMP), calcium ion); [negative regulation of] catalytic activity (ubiquitin-protein transferase/ligase); [negative regulation] of cell death and apoptosis; [positive regulation] of cell motility and movement of cellular components; antigen processing and presentation via the major histocompatibility complex class I (MHC class I) (interferon (IFN) signaling); and transcription mediated by the forkhead box O (FOXO) transcription factor. Molecular functions were primarily binding mechanisms of "sequence-specific" transcriptional DNA cis-regulatory regions, proteins such as transcription (co-)factors or repressors, identical proteins (homo-dimerization), and kinases. Cellular compartments of activity included the extracellular exosome, focal adhesion, and cell-substrate junctions.

For the down-regulated clusters, the biological processes were DNA replication, DNA completion (removal of the flap intermediate); DNA damage response; DNA repair (resolution of D-loop structures, homologous DNA repair (HDR) by homologous recombination (HRR), Double-strand breaks (DSBs)) and [negative regulation of] cell cycle and division (cyclin-dependent G1/S phase transition, initiation of telomere c-strand synthesis and polymerase switching); chromatin organization; and senescence-associated secretory phenotype (SASP).

1.3. Regulation during glucose starvation

Finally, for glucose-starved cells (Fig. 3c), clustering also yielded mostly up-regulated with slightly more down-regulated clusters. For the up-regulated clusters, the biological functions were [positive regulation of] protein metabolism and modification process (post-translational modification (PTM), phosphorylation, proteolysis) and macromolecule biosynthesis; and [positive regulation of] nitrogen compound and nucleic acid metabolism and biosynthesis process (RNA transcription by RNA polymerase II); [negative regulation of] response to stress and stimulus (ER-related, UPR and chaperones, hypoxia, chemicals), organic substances (hormone, cytokine); [positive regulation of] cell localization and transport of vesicles and proteins; [negative regulation of] cell death and apoptotic process and pathway; [negative regulation of] catalytic, transferase, and [positive regulation of] binding activities; antigen processing and presentation; temperature and protein stability homeostasis; [positive regulation of] cell adhesion; and [negative regulation of] transforming growth factor-beta (TGF β) receptor signaling. Molecular functions were mainly binding and homodimerization of "domain-specific" proteins (c-terminal) and binding of enzymes (protein kinase, GTPase and ubiquitin(-like) ligase), anions, misfolded proteins, ribonucleoprotein complexes, and cadherins. Cellular compartments include the cytoplasm (organelle membranes, mitochondria, vesicles and lysosomes, ER and its chaperone complex, Golgi apparatus), protein complexes (transferase, ubiquitin ligase), focal adhesions, and anchoring junctions.

For the down-regulated clusters, biological processes showed DNA methylation and replication; cell cycle and division (regulation of polo-like kinase 1 (PLK1) activity during the G2/M transition, checkpoints, initiation of telomere C-strand synthesis, telomere end packaging); [positive regulation of] chromatin and kinetochore, nuclear fission, microtubule and centriole organization; DNA damage response (Ataxia telangiectasia-mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) upregulation in response to replication stress, DNA damage and telomere stress-induced senescence, oxidative stress-induced senescence); DNA repair (DSBs processing); DNA completion; [positive regulation of] cellular protein, nuclear, RNA localization and chromosome segregation; NOTCH signaling; cholesterol biosynthesis via sterol regulatory element protein (SREBP) gene expression; glycolysis; FOXO-mediated transcription; and SASP.

2. MCF-7 cell line

2.1. Regulation with 2-DG

As with the MCF-7 cell line, comparison of the 2-DG-treated cells with the control (Fig. 4a) yielded only down-regulated clusters, in contrast to what the same comparison yielded for the MDA-MB-231 cell line. The biological processes were metal ion sequestration and zinc ion homeostasis, cell differentiation, and Rho GTPase-activated nicotinamide adenine dinucleotide phosphate (NADPH) oxidases.

2.2. Regulation with metformin

Comparison of MET versus control (Fig. 4b) yielded mostly down-regulated and a few up-regulated clusters. For the up-regulated clusters, the biological properties were as follows: cellular responses to stress (oxygen-containing compound, drug, and steroid hormone); UPR (protein kinase R (PKR)-like ER kinase (PERK)-mediated); ER stress (transcription factor 4 (ATF4)-mediated gene activation, intrinsic apoptotic signaling) and signal transduction by the class mediator p53; cellular autophagy; apoptotic process; cellular differentiation and development; and temperature homeostasis. No molecular function terms were shown due to the paucity of DEGs. Vesicles, lytic vacuoles, and cytoplasm were compartments of activity.

For the down-regulated clusters, the biological functions were: [negative regulation of] mitotic cell cycle and division (checkpoints, G1/S phase transition and associated P53 regulation, ATR in response to replication stress, related to cyclin E); DNA damage response; chromosome segregation; DNA replication (mediated by E2F, up-regulation of the pre-replication complex, DNA unwinding); RNA biosynthesis and transcription via the RNA polymerase II promoter; [negative regulation of] nuclear division, chromosome and cytoskeleton organization; cell population proliferation; and Rho GTPase effectors (formin activation).

2.3. Regulation during glucose starvation

The last comparison, namely glucose starvation versus control (Fig. 4c), resulted in predominantly up-regulated clusters and some down-regulated clusters. For the up-regulated clusters, the biological processes were [positive regulation of] cell response to ER stress (ATF4 activation, intrinsic apoptotic signaling), UPR (Activating transcription factor 6-alpha (ATF6 α) and X-box 1(S) binding protein (XBP1[S]) activate chaperone genes, PERK-mediated response, inositol-requesting enzyme 1 α (IRE1 α)-mediated response), and other stresses and stimuli (organic and cyclic chemicals, lipids, hypoxia, hormones); cell growth; transcription via RNA polymerase II, metabolic process and protein phosphorylation, serine family amino acid biosynthetic process; transmembrane transport of amino acids and hexoses (mediated by ABC family proteins) and cellular localization; regulation of chemical and ion (metals, inorganics, cations) homeostatic process; and FOXO mediated transcription. Molecular functions include transmembrane transport of amino acids and anions, binding of

misfolded proteins, kinases and ubiquitin(-like) ligases, chaperones, homodimerization of proteins, and DNA binding of cofactors and transcription repressors. Cellular compartments include the cytoplasm, the ER chaperone complex, and kinase complexes (cyclin-dependent holoenzyme, serine/threonine).

As for the down-regulated clusters, their biological functions were as follows: [negative regulation of] apoptotic process; mitotic cell cycle (checkpoints, G1/S phase transition and associated transcriptional regulation by P53, and sister chromatid separation); and [positive regulation of] cell population proliferation.

3. Overlapping cell line

To show the functional similarities between the two BC cell types, a gene overlap sham cell line was established. Overlapping up-and down-regulated genes between the two cell lines were identified for each treatment. They were analyzed and cross-referenced in the same manner as the true cell lines.

Overlapping DEGs in the 2-DG treatment revealed too few genes, allowing no enrichment.

3.1. Regulation with metformin

Enrichment with MET resulted primarily in up-regulated clusters and some down-regulated clusters (Fig. 5a). For the up-regulated clusters, the biological processes were a cellular response to UPR, ER stress (intrinsic apoptotic signaling), chemicals, and other stimuli (glucocorticoids, hormones, lipids, chemicals), and RNA biosynthesis and metabolic process. Molecular functions describe the binding of "sequence-specific" DSBs to DNA (transcription regulatory region), and transcription factors. Cellular compartments include the nucleus, chromosome, RNA poly II transcription regulator, and ubiquitin ligase protein complexes.

For the down-regulated clusters, the biological processes were DNA replication, and [negative regulation of the] cell cycle (chromosome segregation, response to DNA damage, cyclin E-associated events during the G1/S transition, Skp1-Cullin-1-F-box (SCF)-mediated degradation of p27/p21, S-phase kinase-associated protein (Skp2)).

3.2. Regulation during glucose starvation

DEGs in the GS primarily resulted in up-regulated clusters (Fig. 5b). For these, the biological processes were as follows: [positive regulation of] ER stress response (ATF4-mediated gene activation, intrinsic apoptotic signaling) and UPR; protein folding in the ER (chaperone activation via ATF6α); [negative regulation of] stimulus response (starvation, steroids, chemicals) ; [negative regulation of] apoptotic signaling pathway; [negative regulation of] protein phosphorylation (cyclin-dependent kinase and MAPK activity) and RNA polymerase II biosynthesis in response to stress; [negative regulation of] transferase activity and catalysis; cell development and differentiation; [positive regulation of] cell localization (ER to cytosol transport, chromosome), epithelial cell motility and migration; cellular redox reaction, chemicals, glucose, and other carbohydrate homeostasis; and FOXO-mediated transcription. Molecular functions describe the binding of misfolded proteins, carbohydrate derivatives, and anions; intramolecular disulfide isomerase activity (S-S bond transposition); chaperone-mediated protein folding; and transmembrane transport of neutral amino acids. Cellular compartments include the cytoplasm, granule (melanosome), ER smooth membrane, chaperone complex, ER quality control compartment, and cell ruffle and leading edge.

For the down-regulated clusters, the biological processes were mitotic cell cycle arrest (p53 regulation of transcription of genes involved in G2 cell cycle arrest, PLK-mediated events, cyclin-associated events), and associated checkpoints and phase transitions; [positive regulation of] chromosome segregation and cell localization; response to DNA damage (G1 mitotic damage, unique transduction by the class mediator p53); cell development and differentiation; and nuclear fission and organelle organization.

4. Preserved functions

The relatively intact functions in the MDA-MB-231 cell line differed between treatments. With all treatments, the G protein-coupled receptor signaling pathway and the detection of chemical stimuli involved in the sensory perception of odor were preserved, the latter being signal transducers and integral components of the cell membrane. For 2-DG, the processes of melanin biosynthesis and [positive regulation of] transmembrane glucose transport were detected. As for GS, nervous system processes (sensory perception of smell) and cell adhesion were described.

For the MCF-7 cell line, actin organization was called, with activities occurring primarily at the plasma membrane, anchor junctions (cell-cell), and the leading edge of the cell. These activities occur specifically at the cell periphery, plasma membrane, extracellular matrix, and collagen trimer. Finally, GS demonstrated a multicellular organization process (sensory odor perception, olfactory signaling pathway), activity in the G protein-coupled receptor signaling pathway, developmental process (nervous system), and chemical stimuli detection. These processes act through the activity of transmembrane signaling receptors and occur at the membrane as integral components.

5. Intersection of DEGs

For this part, genes from the three treatments were intersected and Venn diagrams constructed. These give the number of intersected DEGs in the MDA-MB-231 (Fig. 6a) and MCF-7 (Fig. 6b), and overlap cell lines (Fig. 6c). The gene lists are present in Additional file 4: Table S2, Additional file 5: Table S3, and Additional file 6: Table S4).

5.1. Intersection in the MDA-MB-231 cell line

5.1.1. Common genes between 2-DG and metformin

Starting with the MDA-MB-231 cell line (Fig. 6a), genes common between MET and 2-DG treatments were grouped only into down-regulated clusters. Enrichment only showed activity in pathways related to vitamin metabolism and transport, and bile acid and bile salt synthesis via 24-hydroxycholesterol.

5.1.2. Common genes between 2-DG and glucose starvation

Enrichment of the intersected genes of 2-DG and GS treatments yielded only up-regulated clusters. We cite cellular response to [negative regulation of] organo-nitrogen compound, ER stress (XBP1[S]- and IRE1 α -mediated UPR), stimuli (chemicals, cytokines, cell growth, regulation of insulin-like growth factor (IGF) transport, and uptake by insulin-like growth factor-binding proteins (IGFBP)); protein localization, vesicle-mediated transport, and cell migration; HER2 and TGF β tyrosine kinase signaling; cell development and differentiation; cell adhesion and related regulation; autophagy; phosphorus metabolism and protein PTM; cell death and apoptosis.

5.1.3. Common genes between metformin and glucose starvation

Genes resulting from the intersection of MET and GS yielded predominantly up-regulated clusters with functions such as [negative regulation of] response to stress and stimuli (Lipopolysaccharides (LPS) and lipids); [positive regulation of] innate immune response (tumor necrosis factor (TNF) signaling pathway, NOD-like receptor (NLR) signaling pathway); cell death, apoptosis, and related pathways; cell proliferation; cell development and differentiation, cell aging; and [positive regulation of] cell-cell adhesion. For down-regulated clusters, we have a response to DNA damage stimuli (checkpoint, DSBs processing, p53 class mediator signaling), response to X-ray and UV light; [negative regulation of] DNA replication; [positive regulation of] nitrogen compounds and nucleic acid metabolic process, DNA repair (resolution of D-loop structures by Holliday junction intermediates and synthesis-dependent strand annealing (SDSA), DSBs, HDR), RNA biosynthesis, protein phosphorylation, and ubiquitination; mitotic cell cycle (phase transition, checkpoints, telomere end packaging); organelle nuclear division and chromosome organization; cell division; and SASP.

5.1.4. Common genes between 2-DG, metformin, and glucose starvation

Finally, the genes shared by all treatments mainly exhibited up-regulated clusters with biological functions such as [negative regulation of] stress and stimulus response (chemical, lipid and LPS, glucocorticoid, cytokine, hypoxia, ER and UPR stress, and IFN- α and beta type I and associated pathways); [negative regulation of] catalytic and transferase activity; [negative regulation of] kinase activity (zymogen activation, extracellular signal-regulated kinase $\frac{1}{2}$ (ERK1/2) cascade, RAF-independent MAPK1/3 activation), phosphorus and protein metabolism; biosynthesis of nitrogenous compounds, transcription by RNA polymerase II and ROS production; endogenous peptide antigen presentation by MHC class I; cell population proliferation; [negative regulation of] cell component movement and cell motility; cell migration and differentiation; cell death and apoptosis.

As for the down-regulated clusters, we have the cell cycle (G1/S transition) and DNA repair (HRR).

5.2. Intersection in the MCF-7 cell line

5.2.1. Common genes between 2-DG and metformin

Moving to the MCF-7 cell line (Fig. 6b), few or no genes were found in common between the MET and 2-DG comparison, and thus no significant enrichment resulted.

5.2.2. Common genes between 2-DG and glucose starvation

Comparison of 2-DG and GS treatments yielded only upregulated clusters, namely neurotrophic tropomyosin receptor kinase (NTRKs) signaling.

5.2.3. Common genes between metformin and glucose starvation

Common DEGs between MET and GS treatments yield a predominantly up-regulated enrichment cluster and a down-regulated cluster. For the upregulated, biological functions showed cellular response to chemical stress (oxygen-containing compounds, arsenic), and [positive regulation of] UPR (PERK-mediated), ER stress (ATF response, chaperone activation), corticosteroids, starvation (saccharides, lipids, peptides); cell death and apoptosis, signaling via the class mediator p53; cell proliferation and associated regulation; temperature homeostasis; metabolic processes of amino acids, cyclic and carboxylic, and regulation of transcription from the RNA polymerase II promoter in response to stress; cell differentiation and development; bile acid and bile salt metabolism; transmembrane transport of amino acids; and autophagy.

The down-regulated cluster exhibited cell cycle processes (microtubule-binding).

5.2.4. Common genes between 2-DG, metformin, and glucose starvation

Genes common to all treatments showed only clusters of down-regulation. The cellular processes involved were protein nitrosylation, lipid response, cellular zinc ion homeostasis, cell development and differentiation, [positive regulation of] intrinsic apoptotic signaling pathway, and activity of the cysteine-type endopeptidase involved in the apoptotic process (NF κ B activity), Rho GTPase effectors.

5.3. Intersection in the Overlap cell line

The intersection of treatment genes was only possible between MET and GS (Fig. 6c). One up-regulated cluster resulted from enrichment, having biological functions expressing [positive regulation of] cellular response to ER stress (ATF4 activates genes in response to ER stress) and chemical UPR (PERK regulates gene expression), corticoids, and signaling through apoptotic signaling pathways.

An additional histogram (Fig. 6d) also shows the difference in the number of DEGs between the overlap and the two cell lines.

Discussion

We compared the effect of glucose deprivation on MDA-MB-231 and MCF-7 BC cell lines using microarray data mining methods to establish a database for DEGs, pathways, molecular functions, and cellular compartments. The ultimate goal of this study was to investigate the best way to mimic the beneficial effect of glucose-lowering on the vulnerability of breast cancer cells. Glucose-lowering, or starvation, alone or in combination with conventional treatments, has a substantial effect on the control of cancers, including breast cancer. Two data extraction methods were adopted, each with complementary advantages to the other: the first method uses DAVID to enrich DEGs, yielding clusters of pathway terms (Reactome and KEGG) and gene ontology (Biological Processes, Molecular Functions, and Cell Compartments). The second method uses binary cut clustering, which clusters the same terms except for KEGG. Before focusing on the most significant results, it is worth mentioning that MCF-7 cells were less responsive to glucose deprivation than the MDA-MB-231 cell line. In addition, very few genes were modulated by the 2-DG treatment of MCF-7 cells, resulting in poor enrichment and marginal significance; for this reason, nothing was deemed worthy of discussion. More, starving the cells for glucose had the greatest impact on the genes in terms of modulation, compared with 2-DG and MET treatments.

Intracellular protein aggregation due to slow protein folding is observed under conditions such as lack of nutrients (e.g. glucose) as well as oxygen deprivation, oxidative stress, abnormalities of calcium ion homeostasis, and protein glycosylation [37,38]. Accumulation of misfolded proteins induces ER stress and activates an ER-specific adaptive response, the UPR. The UPR process aims to restore intracellular homeostasis by inducing adaptation pathways related to the activation of three ER transmembrane proteins: IRE1, PERK, and ATF6. These play a role in (i) increasing protein folding capacity via the biosynthesis of chaperones (among others: the 150 kDa oxygen-regulated protein (ORP150), the 78 kDa glucose-regulated protein (GRP78), the 94 kDa glucose-regulated protein (GRP94)), and transcription factors (among others: XBP1, C/EBP homologous protein (CHOP), NF- κ B), (ii) inhibiting protein synthesis, cell growth, and cycle arrest, (iii) increasing misfolded protein degradation, (iv) stimulating antioxidant protection, (v) autophagy, and (vi) apoptosis when stress is severe and intractable [39,40]. Our results showed that MDA-MB-231 cells treated with 2-DG respond to ER stress and activate the UPR pathway leading to apoptosis and autophagy [41,42]. Apoptosis may also be strongly induced by 2-DG mediating the loss of mitochondrial membrane potential (MMP) which leads to mitochondrial damage [43,44]. In addition, protein catabolism by ubiquitination, stimulated by 2-DG and MET, can also induce apoptosis [45]. Interestingly, studies on 2-DG have shown that inhibition of ubiquitination, particularly that of cyclooxygenase-2 (COX-2), promotes angiogenesis, highlighting the anticancer potential of 2-DG [46]. For MCF-7, ER stress and subsequent UPR were also observed with MET and GS. Cell growth was also detected, with concomitant transcription and protein metabolism, requiring transmembrane transport and biosynthesis of amino acids.

We also showed that MET treatment of MDA-MB-231 cells produces ROS. GS and high concentrations of MET (10-40 mM, as seen in our study) sensitizes the highly glycolytic metastatic TNBC cell line, i.e. MDA-MB-231, to apoptosis through increased mitochondrial ROS and MMP disruption [47–50]. As indicated, this activation is due to reduced glucose entry into the hexosamine biosynthetic pathway (HBP), which reduces protein glycosylation levels and leads to sustained stimulation of the UPR and of cell death [51]. This also indicates that ROS play a major role in cancer cell apoptosis in a glucose-depleted environment [52]. MET-treated MCF-7 cells showed increased cellular stress, probably oxidative, and apoptosis. A response to oxygen-containing compounds was observed, consistent with studies describing the generation of oxidative stress (OS) and apoptosis in cells. Sharma and Kumar (2018) detected a decrease in ROS production due, in part, to MET induction of the enzyme superoxide dismutase (SOD). They also concluded that the growth inhibitory effect of MET on BC-derived cells is associated with a reduction in ROS production [53]. Marinello et al. (2020) found that MET treatment-induced lipoperoxidation and reduced total thiol and NO/peroxynitrite levels in cells. MET treatment also interfered with signaling pathways related to OS and cell survival, as treatment increased nuclear p53, consistent with our enrichment results [54]. Of note, reduction in viability mediated by apoptosis has also been demonstrated in our results and studies on the subject [53,55,56]. Dias Lopez et al. (2020) showed that receptor-interacting serine/threonine protein kinase 1 (RIPK1) is important in the cytotoxicity of MET in MCF-7 BC cells. More, they found that MET primarily induces apoptosis; necrosis is rather mostly observed at concentrations of 5 mM and above [55]. This MET-induced apoptosis in BC-derived cells could be caused by ROS-independent mitochondrial dysfunction [53]. Interestingly, the effect of MET appears to specifically target cancer cells with no effect on normal breast epithelial cells [56].

During the cell cycle, deregulation of DNA replication leads to chromosomal alterations, promoting tumorigenesis [57]. We have shown that all three treatments downregulate DNA replication in MDA-MB-231 through suppression of nucleotide metabolism [58]. Inhibition of DNA replication by suppression of minichromosome maintenance protein 5 (MCM5), an important DNA replication initiation factor, has been proven to inhibit tumor growth, increase the sensitivity to cisplatin, as well as induce apoptosis [59]. Throughout the cell cycle, the G1/S phase plays a critical role in the initiation of carcinogenesis through its pivotal regulator, cyclin D1 (CCND1) [60]. In our study and beyond it [61], 2-DG and MET downregulated the G1/S phase transition in the MDA-MB-231 cell line. GS also regulated the cell cycle in MDA-MB-231 cells, specifically PLK1 activity during the G2/M transition. For the MCF-7 cell line, MET treatment-induced inhibition of cell proliferation and associated RNA biosynthesis as well as checking DNA damage, specifically at the p53-regulated G1/S transition. Such proliferation arrest has been described by the majority of MET studies with MCF-7 cells [62–64]. In addition, it became clear in many papers that MET has an antiproliferative effect associated with cell cycle arrest and apoptosis, which is mediated by oxidative stress and AMPK phosphorylation [64,65], among others. In conclusion, MET induces apoptosis and concomitantly blocks cell proliferation and growth in the G1 phase. With the one-week glucose-starved MCF-7 cells, apoptosis was deregulated as well as the cell cycle and, contrary to what was observed with the other treatments, cell proliferation. This deregulation is echoed by other papers describing the same process [66,67]. This state of no apoptosis, no proliferation, and arrest at the G1/S phase transition is suggestive of cellular senescence. This contradicts the theory that UPR promotes a proliferative state due to chaperone accumulation and resistance to chemotherapeutic drugs. In addition, GS has been shown to cause inducible overexpression of ORP150 in breast carcinoma cells. This is due to the ability of chaperones to inhibit senescence, a defense mechanism brought forth by the p53 pathway when oncogenes are stimulated. Thus, when chaperones increase in number, oncogenes are rather activated. The observed prevention of stress-induced apoptotic death may be a consequence of the overexpression of heat shock protein 70 (Hsp70) family of chaperones (ORP150 belongs to this family), which prevents the release of cytochrome c from mitochondria, blocks apoptosome formation by binding to apoptotic protease activating factor (Apaf-1), inhibits the release of apoptosis-

inducing factor (AIF) from mitochondria, and prevents the loss of mitochondrial transmembrane potential. GS has also been shown to increase invasiveness and metastasis of MCF-7 tumor cells. This was explained by stimulation of urokinase plasminogen activator (uPA) and plasmin activity [68].

Compared to normal cells, malignant cells have a higher DNA damage response (DDR), aimed at maintaining genome integrity. They induce DNA repair capacity to cope with DNA damage and to survive. In our study, MET downregulated DNA repair mechanisms in MDA-MB-231 cells, suggesting an increase in TNBC chemosensitivity. MET has been shown to downregulate RAD51 by ubiquitination, a key player in homologous recombination DNA repair, leading to inhibition of the DNA damage repair pathway [69]. ATM and ATR signaling cascades are two key pathways that initiate DDR and are activated in response to DSBs [70]. We showed that GS downregulated ATR activation in response to replication stress, leading to downregulation of DNA repair mechanisms. Therapies inhibiting ATR are currently being tested in early-phase clinical trials in advanced solid tumors [71].

The composition of the extracellular matrix (ECM) changes during breast cancer progression. Our results showed that 2-DG treatment increases collagen, laminin, and proteoglycan organization. Collagen serves as a scaffold for the ECM and its excessive production is a characteristic feature of malignant BC [72]. Studies have shown that collagen gene expression in patients with primary BC is associated with an increased risk of metastasis [73]. In addition, proteoglycans are associated with high invasiveness and progression of BC [74], and the adhesive protein laminin plays an important role in the development of BC. In addition, GS enhances cell adhesion in the MDA-MB-231 cell line. The role of adhesion molecules is to maintain cell-to-cell contact and attachment to the extracellular matrix. Loss of cell adhesion has classically been considered a pro-tumorigenic feature, promoting metastasis and invasion of tumor cells [75]. Adhesion junctions, mediated by E-cadherin and crucial for adhesion between cells, are lost. This may be the result of decreased E-cadherin expression due to methylation of its promoter in TNBC [76].

Our 2-DG-treated MDA-MB-231 cells showed a response to lipids. This has been shown to promote cell migration and invasion, as well as the induction of angiogenesis, as reported by Baenke et al. (2013). The accumulation of energy-rich lipids could provide cancer cells with energy during periods of nutrient depletion [77]. In addition, disruption of lipid metabolism has been linked to breast cancer progression [78]. Therefore, 2-DG did not monitor ECM composition and lipid metabolism.

MET in the MDA-MB-231 cell line increases calcium signaling and activates cAMP signaling. Calcium-based mechanisms have been shown to be crucial for the induction of apoptosis [79], and the role of cAMP as a growth suppressor reduces the metastatic properties of TNBC [80]. In addition, MET enhances the expression of human leukocyte antigen (HLA)-encoded MHC I in the MDA-MB-231 cell line [81]. This is advantageous considering that HLA downregulation is frequently observed in tumors and is thought to correlate with disease progression [82]. In addition, it has been reported that MHC I expression on the tumor membrane is essential for tumor destruction by cytotoxic T lymphocytes (CTL) and the expression of interferon-associated molecules in the BC [83]. IFN signaling was associated with a good response to anthracycline-based chemotherapy.

GS downregulates glycolysis in MDA-MB-231 cells simply due to glucose depletion. In addition, the reduction in the Warburg effect is also due to inhibition of mTOR and regulation of the M2 isozyme of muscle pyruvate kinase (PKM2) [84]. Mitochondrial metabolism and telomerase activity have been shown to decrease by more than 80% and 75%, respectively, under glucose restriction condition [85]. We have reported that GS also downregulates the NOTCH signaling pathway. A recent study demonstrated that NOTCH was required for activation of the sphingosine kinase 1/sphingosine-1-phosphate (SphK1/S1P) pathway to stimulate TNBC cells invasion and migration [86] and that NOTCH activation promotes TNBC formation [87]. TNBC tumors displayed higher activity in cholesterol biosynthesis (CB) than the ER+ tumors [88]. GS down-regulated CB through SREBF, a protein that may be involved in cancer development if an aberrant activation affected it [89]. This deregulation may reduce the increased risk of BC associated with high cholesterol [90], the latter has been shown to promote cell proliferation and migration in TNBC. More, deregulation of cholesterol homeostasis increases cancer cell membrane polarity and mediates apoptosis [91].

To discover the functional similarities between the two different BC cell lines treated with 2-DG, MET, or subjected to GS, an overlap cell line was created. First, no enrichment was possible with overlapping DEGs in 2-DG treatment, indicating that 2-DG had a cell-specific effect. In MDA-MB-231 and MCF-7 cells, MET and GS stimulated ER stress and subsequently UPR activation leading to apoptosis. Inhibition of DNA replication and cell cycle was seen after MET treatment in both cell lines. GS activated protein phosphorylation and degradation by ubiquitination; it also inhibited the cell cycle. Overlap between treatments in the two cell lines was only possible between MET and GS, and the major biological function was the induction of ER stress and activation of ATF4. Thus, our results show that despite the difference between the two BC subtypes, glucose deprivation was able to modulate common pathways and overcome aggressiveness.

Conclusion

In conclusion, our study indicates that the different glucose deprivations have beneficial effects on both BC cell lines. 2-DG appears to be the “gentlest” on the cells. GS for one week in the presence of FBS had the greatest influence on the cells. It should be noted that the MDA-MB-231 cell line responded better than the MCF-7 cell line to all three treatments.

For the MDA-MB-231 cell line, activation of the ER stress response, apoptosis, protein catabolism by ubiquitination, and inhibition of cell cycle and DNA replication were detected after all three treatments. Calcium pathway activation, cAMP, and MHC class I overexpression were induced after MET treatment only. Autophagy, ECM organization, and lipid response were increased after 2-DG treatment only. ROS production and inhibition of DNA repair and SASP mechanisms were reported after MET and GS treatment. Finally, enhancement of cell-cell adhesion, and inhibition of DNA methylation, NOTCH signaling, glycolysis, and cholesterol biosynthesis was observed with GS only.

In the MCF-7 cell line, 2-DG did not result in significant gene modulation. With MET, upregulation of cellular responses to ROS, cellular autophagy, and apoptosis was detected primarily. MET also down-regulated DNA damage response, RNA biosynthesis and transcription, and cell population proliferation. This

effect makes MET a good candidate for cancer co-therapy of ER+ BC cells like MCF-7. In glucose-deprived cells, the enhanced functions included cell growth-related activities (transcription, amino acid import, and protein metabolism). This was associated with deregulation of the apoptotic process and proliferation of the cell population. Both MET and GS upregulated the cell's response to ER stress and the resulting UPR and downregulated the cell cycle and cell division-related processes. As a summary, GS for one week appeared to trigger a senescent state in MCF-7 cells, which is beneficial if stasis was the goal of therapy.

Our results suggest that a combination of MET and GS of the cell lines MDA-MB-231 (a representative model of TNBC) and MCF-7 (a representative model of the HR-positive subtype) is the best approach to restrain both cancer cells. However, longer-term studies need to be performed and the combined effect of food deprivation and other cytotoxic or cytostatic methods also needs to be investigated. Forcing a cancer cell into a state of severe caloric deficit, which could be tolerated by both normal and cancer cells at the same time, may be the best first-line therapy for cancer patients. By stressing the cancer cells, they will reduce their proliferation and enter an energy-saving mode, making the cancers more vulnerable and probably more sensitive to conventional treatments at lower doses, while sparing the side effects on normal cells.

Abbreviations

2-deoxyglucose: 2-DG

78-kDa glucose-regulated protein: GRP78

150 kDa oxygen-regulated protein: ORP150

Activating transcription factor 4: ATF4

Activating transcription factor 6-alpha: ATF6- α

Activating transcription factor: ATF

Adenosine monophosphate: AMP

Adenosine triphosphate: ATP

AMP-activated protein kinase: AMPK

Apoptosis-inducing factor: AIF

Apoptotic protease activating factor: Apaf-1

Ataxia telangiectasia-mutated: ATM

Ataxia-telangiectasia and Rad3-related: ATR

Basal-like 1: BL1

Basal-like 2: BL2

Breast cancer stem cells: BCSCs

Breast Cancer: BC

c-Jun N-terminal kinase: JNK

C/EBP homologous protein: CHOP

Cholesterol biosynthesis: CB

Cyclic adenosine monophosphate: cAMP

Cyclin D1: CCND1

Cytotoxic T lymphocytes: CTLs

Database for annotation, visualization, and integrated discovery: DAVID

Differentially expressed genes: DEGs

Double strand breaks: DSBs

Double strand: ds

Endoplasmic reticulum protein kinase R-like kinase: PERK

Endoplasmic reticulum: ER

Inositol requiring enzyme 1 α : IRE1 α

Estrogen receptor: ER+.

Extracellular matrix: ECM

Extracellular signal-regulated kinase 1/2: ERK1/2

F-box 2 protein associated with the S-phase kinase: Skp2

Fold change: FC

Forkhead box O transcription factor: FOXO

Gene Ontology: GO

Glucose starvation: GS

Glucose-regulated endoplasmic reticulum chaperone protein 94: GRP94

Glutathione peroxidase 4: GPX4

Glycosaminoglycans: GAGs

Heat shock protein 70: Hsp70

Hexokinase 2: HK2

Hexosamine biosynthetic pathway: BPH

Homologous DNA repair: HDR

Homologous recombination: HRR

Hormone receptor: HR

Human epidermal growth factor receptor 2: HER2

Human leukocyte antigen: HLA

Immunomodulators: IM

Insulin-like growth factor 1: IGF-1

Insulin-like growth factor binding proteins: IGFBP

Insulin-like growth factor: IGF

Interferon: IFN

Interleukin: IL

Kyoto Encyclopedia of Genes and Genomes: KEGG

Lipopolysaccharides: LPS

Luminal androgen receptor: LAR

Maintenance proteins of minichromosome 5: MCM5

Major histocompatibility complex class I: MHC class I

Mammalian target of rapamycin: mTOR

Mesenchymal stem-like: MSL

Mesenchymal: M

Metformin: MET

Mitochondrial membrane potential: MMP

Mitogen-activated protein kinase: MAPK

Neurotrophic tropomyosin receptor kinases: NTRKs

Nicotinamide adenine dinucleotide phosphate oxidase: NADPH

NOD type receptor: NLR

Non-homologous end-joining: NHEJ

Nuclear factor-kappa B: NFkB

Oxidative phosphorylation: OXPHOS

Oxidative stress: OS

Phosphoinositide 3-kinase: PI3K

Polo-like kinase 1: PLK1

Post-translational modification: PTM

Progesterone receptor: PR+.

Protein kinase B: AKT

Protein kinase C: PKcs

Protein kinase R: PKR

Pyruvate kinase muscle isozyme M2: PKM2

Reactive oxygen species: ROS

Receptor-interacting protein kinase 1 (serine/threonine): RIPK1

Response to DNA damage: DDR

Response to unfolded proteins: UPR

Significance Analysis of Microarrays: SAM

Skp1-Cullin-1-F-box: SCF

Small nuclear RNA: snRNA

Sphingosine kinase 1/ sphingosine-1-phosphate: SphK1/S1P

Sterol regulatory element binding protein gene: SREBP

Superoxide dismutase: SOD

Synthesis-dependent strand annealing: SDSA

The secretory phenotype associated with senescence: SASP

Toll-like receptor: TLR

Transcriptome Analysis Console: TAC

Transfer ribonucleic acid: tRNA

Transforming growth factor beta: TGFβ

Triple-negative breast cancer: TNBC

Tumor necrosis factor: TNF

Tumor protein p53: p53

Urokinase plasminogen activator: uPA

X-box 1(S) binding protein: XBP1(S)

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

RA performed the experiments, participated in the design of the project, analyzed the microarray data, and wrote the manuscript. CEH participated in data analysis, and was a major contributor in writing the manuscript. RT designed the project and participated in manuscript revision. REH participated in the experimental work (cell culture and treatments), and made substantial contributions to the manuscript's sections editing. GH supervised the study, provided guidance and edited the manuscript. All authors read and approved the final manuscript.

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Footnotes

Not applicable

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Figures

Figure 1

A simplified schematization of the partitioning of DEGs

Figure 6

The intersection of DEGs in MDA-MB-231, MCF-7, and “overlap” cell lines following the treatment with 2-DG, MET, and GS. Venn diagrams displaying the number of upregulated and downregulated DEGs determined using TAC and SAM in (a) MDA-MB-231, (b) MCF-7, and (c) “overlap” cell lines. (d) Histograms presenting the difference in DEGs of the cell lines. Results from SAM are shown in parenthesis. Statistical criteria: p-value <0.05, FC <-2 or >2

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