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Original article

Effects of neoadjuvant therapies on genetic regulation of targeted pathways in ER+ primary ductal breast carcinoma: A meta-analysis of microarray datasets

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ABSTRACT

Breast cancer arises as a result of multiple interactions between environmental and genetic factors. Conventionally, breast cancer is treated based on histopathological and clinical features. DNA technologies like the human genome microarray are now partially integrated into clinical practice and are used for developing new "personalized medicines" and "pharmacogenetics" for improving the efficiency and safety of cancer medications. We investigated the effects of four established therapies—for ER+ ductal breast cancer—on the differential gene expression. The therapies included single agent tamoxifen, two-agent docetaxel and capecitabine, or combined three-agents CAF (cyclophosphamide, doxorubicin, and fluorouracil) and CMF (cyclophosphamide, methotrexate, and fluorouracil). Genevestigator 8.1.0 was used to compare five datasets from patients with infiltrating ductal carcinoma, untreated or treated with selected drugs, to those from the healthy control. We identified 74 differentially expressed genes involved in three pathways, i.e., apoptosis (extrinsic and intrinsic), oxidative signaling, and PI3K/Akt signaling. The treatments affected the expression of apoptotic genes (*TNFRSF10B* [*TRAIL*], *FAS*, *CASP3/6/7/8*, *PMAIP1* [*NOXA*], *BNIP3L*, *BNIP3*, *BCL2A1*, and *BCL2*), the oxidative stress-related genes (*NOX4*, *XDH*, *MAOA*, *GSR*, *GPX3*, and *SOD3*), and the PI3K/Akt pathway gene (*ERBB2* [*HER2*]). Breast cancer treatments are complex with varying drug responses and efficacy among patients. This necessitates identifying novel biomarkers for predicting the drug response, using available data and new technologies. *GSR*, *NOX4*, *CASP3*, and *ERBB2* are potential biomarkers for predicting the treatment response in primary ER+ ductal breast carcinoma.

Abbreviations: BC, breast cancer; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; ROS, reactive oxygen species; OH●, hydroxyl radical; H_2O_2, hydrogen peroxide; Bcl2, B-cell lymphoma 2; PI3K/Akt, phosphatidylinositol 3-kinase/protein kinase B; Bax, Bcl-2-associated X; FU, fluorouracil; TS, thymidylate synthase; DC, docetaxel and capecitabine; TMX, tamoxifen; TGF-α/β, transforming growth factor alpha/beta; IGF-1, insulin-like growth factor-1; PM, personalized medicine; CAF, cyclophosphamide, doxorubicin, and fluorouracil; CMF, cyclophosphamide, methotrexate, and fluorouracil; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; FC, fold-change.

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1. Introduction

Breast cancer (BC) arises as a result of multiple interactions between environmental and genetic factors. Globally, 25% of the women are diagnosed with BC, and this percentage is projected to increase in the next 20 years (Meeske et al., 2007); it is the most common type of cancer and the second leading cause of death in women (Wyld et al., 2017). Inefficiency in diagnosis and early detection of BC is responsible for the high mortality rate in women in the Middle East. Importantly, BC is diagnosed at much later stages in pre-menopausal women in Saudi Arabia than in countries such as the United States of America (Alshareef et al., 2020). BC is classified as non-invasive or invasive, based on the histology and location. Non-invasive BC does not spread beyond the breast tissues and it is classified as, *in situ* lobular carcinoma and *in situ* ductal carcinoma. Invasive BC is the most diagnosed and is frequently metastatic; it is classified into six types of carcinomas, i.e., infiltrating ductal, tubular, invasive lobular, colloid, inflammatory, and medullary. At the molecular level, BC is classified based on the expression of estrogen receptor (ER), progesterone receptor (PR), or the human epidermal growth factor receptor 2 (HER2), and is further divided into four types, luminal A, luminal B, HER2, and triple negative (Singh and Khan, 2019).

Cancers arise as a result of imbalance between the survival and cell death pathways (Figs. 1 and 2) (Chen et al., 2016). Several of these imbalances are directly linked to carcinogenesis (Fakhri et al., 2019; Ochwang'i et al., 2014). The mechanisms underlying cancer initiation, progression, and pathophysiology involve dysregulated apoptotic and oxidative stress pathways (Nouri et al., 2020). In addition, the PI3K/AKT pathway also plays a crucial role in the regulation of various cellular functions, including transcription, protein synthesis, metabolism, growth, proliferation, and survival (Jiang et al., 2020). When the balance between cell division and growth is disturbed, the cells either become cancerous or undergo apoptosis. Rapid changes in the balance increase the chance of oncogenic alterations in the proteins and pathways regulating cell development, proliferation, and growth (Fresno Vara et al., 2004).

Reactive oxygen species (ROS) are free radicals formed as a byproduct of normal cellular metabolism and they play an important role in cell signaling (Alpay et al., 2015). ROS are associated with cancer initiation and development; however, they can also induce apoptosis, which makes them potential therapeutic candidates (Khan et al., 2010; Woo et al., 2013; Dai et al., 2017; Kim et al., 2018). ROS and oxidative stress-modulating therapeutics can have varying effects ranging from ROS-induced cell death to antioxidant inhibition in malignant cancers (Gorrini et al., 2013; Zou et al., 2017). In cancer cells, hyperproliferation results in uncontrolled metabolism, which further increases the ROS levels (Storz, 2017). Elevation in ROS levels above the cytotoxic threshold results in impaired redox homeostasis and consequently, apoptosis of cancerous cells (Raza et al., 2017). Oxidative stress is induced when increased ROS generation results in elevated levels of free radicals (Forcados et al., 2016). The reactive species are mostly generated in the mitochondria. The interaction between O_2 and electrons during ATP generation results in the formation of superoxide anion, which interacts with other molecules such as Fe^{2+}, leading to the generation of reactive species such as hydroxyl radical (OH●), organic peroxides, and hydrogen peroxide (H_2O_2) (Pisoschi and Pop, 2015). DNA damage, induced by oxidative stress, promotes carcinogenesis and there is a positive correlation between oxidative stress and BC in postmenopausal women (Fortner et al., 2013; Loft et al., 2013; Rossner et al., 2006). Oxidative stress also exerts beneficial effects such as apoptosis induction and senescence, both of which play an essential role in preventing cancer before the onset of menopause (Nemoto and Finkel, 2004).

Apoptosis, also known as programed cell death, is a natural mechanism for eliminating infected, injured, or aged cells from the system (Hirsova and Gores, 2015; Reed, 2000). It plays an essential role in both homeostasis and development (Hassan et al., 2014). The apoptotic pathway is activated through intracellular and extracellular signaling, initiating either the intrinsic (mitochondrial) or extrinsic (death receptor) pathways (Zaman et al., 2014). Cancer cells escape apoptosis through different mechanisms and the deflection from the normal pathways results in either pro-survival or pro-apoptotic regulation. Pro-survival genes are potential oncogenes, and mutations in these genes upregulate their expression (Adams and Cory, 1998); while pro-apoptotic genes serve as tumor suppressors. The expression of anti-apoptotic and pro-apoptotic factors is dysregulated in the cancer cells; the expression of anti-apoptotic B-cell lymphoma 2 (Bcl2) is upregulated in approximately half of the human cancers (Yip and Reed, 2008).

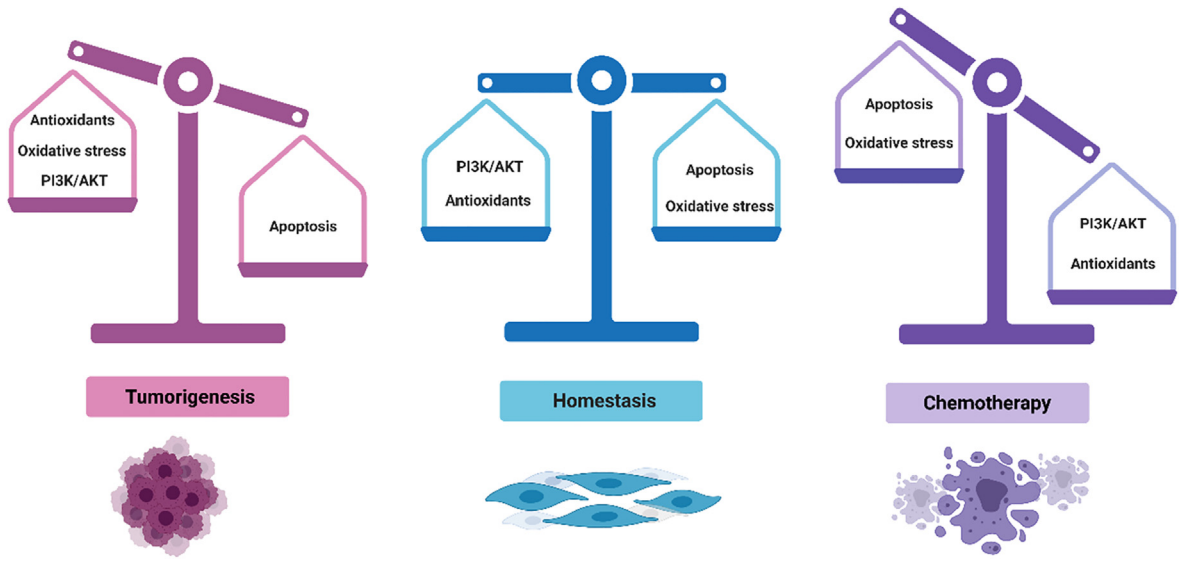

Fig. 1. Balance between apoptosis, oxidative stress, antioxidants, and PI3K/AKT ROS.

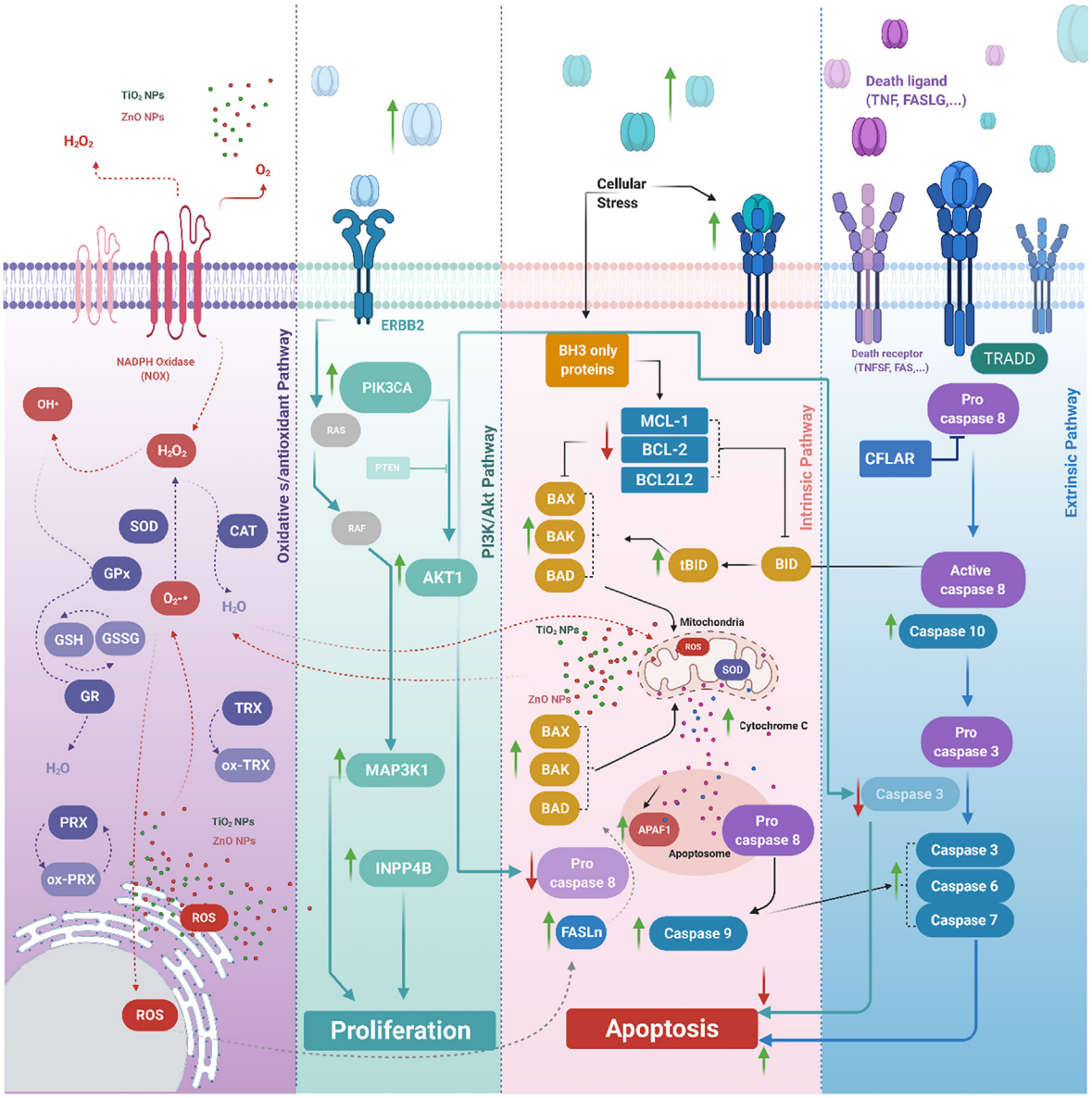

Fig. 2. Schematic diagram of genes involved in apoptosis, oxidative/antioxidant, and PI3K/Akt pathways.

Phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling plays a central role in the regulation of phenomenon, such as metabolism, proliferation, and survival (Yang et al., 2016). Mutations in genes encoding intermediaries of the PI3K/Akt pathway result in tumorigenesis (Mayer and Arteaga, 2016), inhibiting apoptosis in tumor cells (Zheng et al., 2004). The pro-apoptotic protein, Bcl-2-associated X (Bax) induces the release of mitochondrial cytochrome *c* in response to apoptotic stimuli. This is inhibited by the activation of the PI3K/Akt pathway (Tsuruta et al., 2002); and therefore, this pathway provides multiple molecular targets for therapy. However, specific research is required for determining the pivotal target that will provide the most therapeutic effects (Mayer and Arteaga, 2016).

Traditional BC treatments include hormonal therapy and chemotherapy, which are nonspecifically used to treat all women diagnosed with BC. Most BC treatments trigger multiple pathways and induce cell death by activating extrinsic (includes the death receptor) and intrinsic (mitochondrial) apoptotic pathways. Some BC drugs target intracellular redox signaling for inhibiting ROS-induced cancer progression and inducing apoptotic signaling pathways (Shacter et al., 2000).

A new generation of chemotherapeutic drugs (e.g. the semisynthetic taxane, docetaxel) and monoclonal antibodies including trastuzumab, have been developed. Docetaxel inhibits microtubular polymerization by binding to β-tubulin rather than to guanosine triphosphate under normal conditions. This interferes with normal mitotic processes leading to cell arrest in G2/M phase of the cell cycle, followed by apoptosis (Pienta, 2001). Capecitabine is a fluorouracil (FU) pro-drug absorbed in the gastrointestinal tract and converted to 5-FU by thymidine phosphorylase in the liver. This enzyme is abundantly expressed in the tumor tissues and in the plasma of cancer patients (Mikhail et al., 2010). 5-FU affects nucleic acid synthesis by inhibiting thymidylate synthase, which is important for DNA synthesis and repair (Longley et al., 2003). Combination therapy using both docetaxel and capecitabine (DC) exerts improved effects, because docetaxel upregulates thymidine phosphorylase, thereby increasing the conversion of capecitabine to 5-FU in tumor tissues (O'Shaughnessy, 2003).

Tamoxifen (TMX) is an estrogen inhibitor, which binds to estrogen receptors and blocks cell proliferation, growth, and division. TMX downregulates the expression of transforming growth factor alpha (TGF-α) and insulin-like growth factor-1 (IGF-1) and upregulates the expression TGF beta (TGF-β), a growth suppressor that accumulates in the G1/G2 phases, thereby causing cell cycle arrest (McKeon, 1997; Ichikawa et al., 2008; Li et al., 2017; Lykkesfeldt et al., 1984).

Cyclophosphamide is an alkylating agent sub-classified as oxazaphosphorine (a non-cell-cycle-specific agent). Following metabolic activation, it inhibits protein synthesis through DNA- and RNA-alkylation and crosslinking, resulting in cell death (Fleming, 1997; Mills et al., 2019; Korkmaz et al., 2007). In the liver, cyclophosphamide is converted into phosphoramide mustard and acrolein by the hepatic enzyme, cytochrome *P*-450. The phosphoramide mustard forms cross-linkages between and within contiguous DNA strands at the guanine N-7 position (Colvin, 1999). It selectively targets cancer cells, owing to their high expression of phosphamidase (Emadi et al., 2009).

Doxorubicin is an anthracycline drug extracted in the 1970s from *Streptomyces peucetius* var. *caesius* and is used to treat various cancers, including multiple myeloma, Hodgkin's and non-Hodgkin's lymphomas, and lung, breast, gastric, ovarian, sarcoma, thyroid, and pediatric cancers (Arcamone et al., 1969; Cortes-Funes and Coronado, 2007; Weiss, 1992). Two mechanisms of action have been proposed for doxorubicin, i.e., DNA topoisomerase-II mediated repair disruption through intercalation with DNA; and ROS-induced damage to protein, DNA, and cellular membranes (Gewirtz, 1999). Doxorubicin leads to cell cycle arrest at the G2/M phase, leading to cell death (Kim et al., 2009).

Methotrexate indirectly blocks cell division by inhibiting dihydrofolate reductase. This enzyme converts dihydrofolate into tetrahydrofolate, a vital coenzyme involved in several transmethylation reactions during purine and pyrimidine synthesis, and therefore essential for DNA synthesis, repair, and replication (Genestier et al., 2000; Tian and Cronstein, 2007). Methotrexate leads to cell cycle arrest in the S phase (Yamauchi et al., 2005; Tsurusawa et al., 1990).

The choice of treatment is based on the histopathological and TNM features, and the expression of ER, PR, and HER2 in the tumor, all of which are used predict the tumor aggression, probability of recurrence/relapse, and the treatment outcomes. Technologies, such as high-throughput sequencing, next-generation sequencing, transcriptome analysis, and microarray technology are partially integrated into clinical practice, and are used for developing "personalized medicine (PM)" and "pharmacogenetics" that can improve the efficiency and safety of cancer medication (Reuter et al., 2015).

PM is aimed at tailoring therapy to enable each patient to receive optimal treatment (high efficacy and safety) for better healthcare at reduced cost (Vogenberg et al., 2010). Genome sequencing and other large scale omics help identify specific therapeutic targets, thereby improving personalized diagnostics and treatments (Collins and Workman, 2006). Inter-patient pharmacokinetic differences are likely contributors to drug resistance; therefore, PM cannot rely solely on tumor cell characteristics, but requires an individual Pharmacological Audit Trail (Yap et al., 2010). This framework contains a set of questions raised during the discovery and development of antineoplastic drugs (Banerji and Workman, 2016), including (i) determination of patient population, (ii) characteristics of drug pharmacokinetics, (iii) description of drug pharmacodynamics, (iv) prediction of the tumor response at the transitional time point, (v) assessment of the tumor response at the treatment end-point, and (vi) tumor resistance pattern (Rossanese et al., 2016).

Pharmacogenetics and PMs are promising strategies for managing BC. Our study evaluates the effects of therapeutic drugs (single agent, TMX; two-agent, DC; or combined three-agents, CAF (cyclophosphamide, doxorubicin, and fluorouracil) or CMF (cyclophosphamide, methotrexate, and fluorouracil))—currently used for treating ER+ ductal breast carcinoma—on the gene expression profiles. We analyzed the relationship between the expression patterns and the established therapeutic pathways related to cellular growth and death. We discuss on the most effective drug targets, based on the mechanism and gene expression profiles.

2. Materials and methods

2.1. Study design

This study was conducted in agreement with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009). The PRISMA flow diagram of the datasets included in this study is shown in Supplementary Fig. S1.

2.2. Drugs

The seven drugs studied in this analysis (docetaxel, capecitabine, TMX, cyclophosphamide, methotrexate, doxorubicin, and fluorouracil) were approved as BC treatments by the Food and Drug Administration and validated by both the National Cancer Institute (https://www.cancer.gov/) and American Cancer Society (https://www.cancer.org/). The DrugBank database (https://www.drugbank.ca/) was used to collect drug IDs and information. These drugs were chosen for both their mutual targeting of genes and specific cell cycle phase inhibition. The information and characteristics of the included drugs are presented in Table S1 and the chemical structures are illustrated in Supplementary Fig. S2. Briefly, 141 records were identified from the database, of which 69 were screened. Among them, 64 were excluded for: (1) not presenting with infiltrating ductal carcinoma, (2) use of drugs outside of the aforementioned list, (3) not using the GPL570 microarray platform, (4) pregnancy, or (5) presence of metastasis. Therefore, five eligible records were included in the qualitative and quantitative synthesis.

2.3. Pathways and genes

The drugs were administered as either a single-, two-, or three-agent therapy, and the intracellular signaling pathways were evaluated. The three most affected signaling pathways were apoptosis (extrinsic and intrinsic), antioxidant pathways, and the PI3K/Akt pathway. The expression pattern of 74 genes was investigated: 12 genes encode for death receptor proteins; 11, for caspase proteins; 16, for pro-apoptotic proteins; 6, for anti-apoptotic proteins; 7, for reactive species; 14, for antioxidants; and 8, for PI3K/Akt genes. (Table S2).

2.4. Gene expression data source, tumor types, control samples, and eligibility criteria

Genevestigator 8.1.0 (Nebion AG, Zurich, Switzerland) was used to evaluate gene expression. The datasets satisfied the following criteria: i) use of the Affymetrix Human Genome U133 Plus 2.0 Array platform, ii) gene expression data for patients with infiltrating ductal carcinoma either treated with only selected drugs or untreated for comparison with healthy breast tissues (control). The Genevestigator dataset was downloaded in November 2020,

and 529 samples were analyzed (Table S3). The demographic overview of the clinical and molecular characteristics of the datasets is summarized in Table S4.

2.5. Statistical analysis

GraphPad Prism 9.0 (San Diego, CA, USA) was used to perform all statistical analyses. Data are expressed as mean ± SD. Fold-change (FC) was calculated for each gene per treatment (compared to either healthy tissue and/or BC), using the signal intensity $\log_2$ value. FC > 1.5 was considered significant. Unpaired *t*-test was used to compare the mean of each treatment and/or tumor with the mean of the healthy control. Differences were considered significant at $p < 0.05$. Chord diagrams were used to construct a drug-affected gene network to demonstrate the connection between each drug and pharmacogene, for each investigated pathway, using Chordial (Maria Nattestad, San Francisco, CA, USA). Venn Painter 1.2.0 (University of Toronto, Canada) was used to draw all the classic Venn diagrams, for identifying the candidate genes (Lin et al., 2016).

3. Results

The expression of genes in the target pathways was compared across several tissue types with healthy tissue as a control, untreated tissues of BC, and tissues treated with different drugs including TMX, CAF, CMF, and a DC combination.

3.1. Evaluating the influence of the drugs on three most affected pathways

3.1.1. Influences on apoptosis pathways

3.1.1.1. The expression of death receptor and ligand genes. The datasets were assessed for differential expression of genes associated with the death receptor (Supplementary Figs. S3A and S4); these included 12 genes, i.e., *FAS*, *FASLG*, *TNFRSF10A*, *TNFRSF10B*, *TNFRSF10C*, *TNFRSF10D*, *TNFRSF11B*, *TNFSF10*, *TNFRSF1A*, *TNF*, *FADD,* and *CFLAR*. The DC-treated tissues exhibited significant upregulation in the expression of *TNFRSF10B* (*TRAIL*), compared to the healthy tissue (FC = 1.642, $p < 0.0001$) and BC tissue(FC = 1.73, $p < 0.0001$) and significant upregulation of *FAS* expression, when compared to the BC tissue (FC = 2.28, and $p < 0.0001$); while, *FAS* expression was significantly downregulated in BC tissue, when compared to that in the healthy tissue (FC = -1.56).

3.1.1.2. The expression of caspases. The caspase gene group (Supplementary Figs. S3B and S4) comprised 11 genes (*CASP1*, *CASP2*, *CASP3*, *CASP4*, *CASP5*, *CASP6*, *CASP7*, *CASP8*, *CASP9*, *CASP10,* and *CASP14*). Significant changes were detected in the expression of four caspase genes (*CASP3*, *CASP6*, *CASP7*, and *CASP8).* The expression of *CASP3* was significantly upregulated by all drug treatments ($p < 0.0001$), compared to that in the healthy tissue, with the highest FC = 2.37 in TMX-treated tissue, FC = 1.78 in CMF-treated tissue, FC = 1.75 in CAF-treated tissue, and FC = 1.65 in DC-treated tissue. The expression of *CASP6* was influenced by both TMX and CMF, resulting in significant upregulation with FC = 1.83; $p < 0.0001$ and 1.58; $p < 0.0001$, respectively, when compared to that in the healthy tissue. The expression of *CASP7* was significantly upregulated in both TMX- and DC-treated tissues, when compared to that in healthy tissue (FC = 1.95, and FC = 1.58, respectively, $p < 0.0001$ for both), while it was only upregulated in TMX-treated tissues (compared to that in BC tissue; FC = 1.52, $p < 0.0001$). The expression of *CASP8* was significantly upregulated in both DC and CAF-treated tissue (compared to that in healthy tissue; FC = 1.54 and 1.68, respectively, $p < 0.0001$ for both).

3.1.1.3. The expression of pro-apoptotic genes. The pro-apoptotic gene group (Supplementary Figs. S3C and S4) included 16 genes (*BAX*, *BAK1*, *BOK*, *BID*, *BCL2L11*, *BMF*, *BAD*, *BIK*, *HRK*, *PMAIP1*, *BNIP3*, *BNIP3L*, *BCL2L14*, *BBC3*, *BCL2L12,* and *BCL2L13*). Only three genes were significantly and differentially expressed following the treatments, i.e., *PMAIP1*, *BNIP3*, and *BNIP3L*. The expression of *PMAIP1* was upregulated in all treatments except DC treatment (compared to that in healthy tissue). The *PMAIP1* expression FC's for TMX-, CAF-, CMF-treated tissues were FC = 2.241 ($p < 0.0001$), 1.547 ($p < 0.001$), and 1.541 ($p < 0.0001$), respectively. The expression of *PMAIP1* was significantly upregulated in treated tissues, when compared to that in the BC tissue (FC = 2.29, 1.599, and 1.594, for TMX, CAF, and CMF, respectively, $p < 0.0001$ for all). The expression of *BNIP3* was significantly upregulated in the TMX-treated tissue, compared to that in the healthy tissue (FC = 2.18, $p < 0.0001$). The expression of *BNIP3L* was downregulated in the BC tissue, compared to that in healthy tissue (FC = -1.537, $p < 0.0001$), and significantly downregulated in DC, CAF, and CMF treatments compared to that in healthy tissue (FC = -1.973, −1.854, and −1.955, respectively, $p < 0.0001$ for all). TMX treatment had no significant effect on the expression of *BNIP3L*. The expression of *BCL2L14* was significantly upregulated in the DC-treated tissue, compared to that in the BC tissue (FC = 1.5607, $p < 0.0001$).

3.1.1.4. The expression of anti-apoptotic genes. The anti-apoptotic group (Supplementary Figs. S3D and S4) included 6 genes (*BCL2*, *MCL1*, *BCL2L1*, *BCL2L2*, *BCL2A1,* and *BCL2L10*). Only *BCL2* and *BCL2A1* exhibited significant differential expressions among the treated, BC, and healthy tissues. The expression of *BCL2* was downregulated in the BC tissue, compared to that in healthy tissues FC = -1.927, $p < 0.0001$), while it was upregulated in the TMX- and DC-treated tissues, compared to that in BC tissue (FC = 3.020, and FC = 1.838, respectively, $p < 0.0001$ for both). The expression of *BCL2A1* was significantly upregulated in DC-, CAF-, and CMF-treated tissues, compared to that in the healthy tissue (FC = 1.546 ($p < 0.01$), FC = 1.664 ($p < 0.001$), and FC = 1.509 ($p < 0.01$), respectively). In addition, the expression of *BCL2A1* was significantly upregulated in the CAF-treated tissue compared to that in the BC tissue (FC = 1.603, $p < 0.0001$).

3.1.2. Influences on the oxidative stress and antioxidant pathways

3.1.2.1. The expression of ROS genes. The ROS group (Supplementary Figs. S5A and S6) consisted of 7 genes (*NOX1*, *NOX3*, *NOX4*, *NOX5*, *XDH*, *CYBB,* and *MAOA*). Among these genes, only *NOX4*, *XDH*, and *MAOA* were differentially expressed. The expression of *NOX4* was significantly upregulated in BC tissue, compared to that in the healthy tissue (FC = 2.869, $p < 0.001$); It was significantly upregulated in all the treated tissues (compared to that in the healthy tissue; FC = 1.919, 2.357, 2.288, and 1.907, respectively for TMX, DC, CAF, and CMF, $p < 0.001$ for all). The expression of *XDH* was significantly downregulated in the BC tissue, compared to that in normal tissue (FC = -2.197, $p < 0.001$); it was also downregulated in the TMX-, CAF-, and CMF-treated tissues, compared to that in healthy tissues (FC = -3.105, −2.710, and −2.596, respectively, $p < 0.001$ for all), while there was no significant difference in the DC-treated tissues. The expression of *MAOA* was significantly downregulated in the BC tissue, compared to that in healthy tissue (FC = -2.474, $p < 0.0001$), and in the CAF- and CMF-treated tissues, compared to that in healthy tissue (FC = -1.767, and −1.822, respectively, $p < 0.001$ for both).

3.1.2.2. The expression of antioxidant genes. The antioxidant gene group (Supplementary Figs. S5B and S6) contained 14 genes (*TXN2*, *TXNRD1*, *TXNRD2*, *TXN*, *NFE2L2*, *SOD1*, *SOD2*, *SOD3*, *CAT*, *GPX1*, *GPX3*, *PRDX1*, *PRRX1*, and *GSR*). Eight among them, *TXNRD1*, *TXN*, *SOD1*, *SOD3*, *CAT, GPX3*, and *GSR,* exhibited differential expres-

sion. The expression of *TXNRD1* was upregulated in the DC-treated tissue (compared to that in the healthy tissue; FC = 1.673, $p < 0.0001$). The expression of *TXN* in the TMX-treated tissue was upregulated (compared to that in the healthy tissue; FC = 2.22, $p < 0.0001$), and downregulated compared to that in the BC tissue (FC = -1.791, $p < 0.0001$). The expression of *SOD1* was upregulated in the TMX-treated tissue (compared to that in the BC tissue; FC = 1.595, $p < 0.0001$). The expression of *SOD3* was downregulated in the TMX-, CAF-, and CMF-treated tissues, compared to that in the healthy tissue (FC = -1.576, −1.918, and −1.872, respectively, $p < 0.0001$ for all). The expression of both *CAT* and *GPX1* was downregulated in DC-treated tissues, compared to that in BC tissues (FC = -1.624, and −1.568, $p < 0.0001$ for both). The expression of *GPX3* was significantly downregulated in BC tissue (FC = -1.665, $p < 0.001$), and in the TMX-, CAF-, and CMF-treated treated tissues (FC = -2.257, −2.243, and −2.168, respectively, $p < 0.0001$ for all), compared to that in normal tissues. The expression of *GSR* was upregulated in the TMX-, DC-, CAF-, and CMF- treated tissues, compared to that in the normal tissue (FC = 2.202, 2.264, 1.683, and 1.615, respectively, $p < 0.0001$ for all).

3.1.3. Influences on the PI3K/Akt pathway

3.1.3.1. The expression of PI3K/Akt genes. The PI3K/Akt group (Supplementary Figs. S7 and S8) consisted of 8 genes (*ERBB2*, *PIK3CA*, *PDK1*, *AKT1*, *PTEN*, *PIK3R1*, *MAP3K1*, and *INPP4B*). Five genes, *ERBB2*, *PTEN*, *PIK3R1*, *MAP3K1*, and *INPP4B*, were differentially expressed. The expression of *ERBB2* was significantly upregulated in BC (compared to that in the healthy tissue; FC = 2.627, $p < 0.0001$), while it was significantly downregulated in DC-treated tissue (compared to that in the healthy tissue; FC = -1.606, $p < 0.0001$). The expression of *ERBB2* was significantly downregulated in the TMX-, DC-, CAF-, and CMF-treated tissue (compared to that in BC tissue; FC = -2.562, −4.223 ($p < 0.01$), −2.108 ($p < 0.0001$), and −2.213 ($p < 0.0001$), respectively). The expression of *PTEN* was significantly downregulated in the BC tissue (compared to that in the healthy tissue; FC = -1.518, $p < 0.0001$), while it was significantly upregulated in the DC-treated tissue (compared to that in the BC tissue; FC = 2.137, $p < 0.0001$). The expression of *PIK3R1* was downregulated in the TMX-treated tissue (compared to that in healthy tissue; FC = -1.699, $p < 0.0001$). The expression of *MAP3K1* was upregulated in the TMX-treated tissue (compared to that in the BC tissue; FC = -1.969, $p < 0.0001$). The expression of *INPP4B* was significantly upregulated in TMX-treated tissues (compared to that in both BC and healthy tissues; FC = 1.709, and 1.961, respectively, $p < 0.0001$ for both).

3.2. Pattern of gene expression associated each therapy

3.2.1. TMX xxxx

The expression of 13 genes was altered in response to TMX treatment, compared to that in healthy tissue; 9 were significantly upregulated, while 4 were downregulated (Supplementary Fig. S9B). This pertains to 17.57% of affected genes for TMX-treatment: 6.76% genes related to the apoptosis pathway, 8.11% to the antioxidant pathway, and 2.70% genes to the PI3K/Akt pathway (Fig. 3A). The expression of 8 genes was significantly altered, when compared to that in BC tissues; 7 significantly upregulated, and 1 downregulated (Supplementary Fig. S9C). This pertains to 10.8% affected genes for the TMX-treated tissue, compared to that in the BC tissue, with 4.05% genes related to the apoptosis pathway, 2.70% genes to the antioxidant pathway, and 4.05% genes to the PI3K/Akt pathway (Fig. 3B).

3.2.2. DC xxxx

The expression of 10 genes was altered in response to DC treatment, compared to that in healthy tissue; 8 were significantly upregulated, and 2 downregulated (Supplementary Fig. S9D). This pertains to 13.51% affected genes for the DC-treated tissue, compared to that in the control, with 8.11% genes related to the apoptosis pathway, 4.05% to the antioxidant pathway, and 1.35% genes to the PI3K/Akt pathway (Fig. 3C). The expression of 8 genes was significantly affected (compared to that in BC tissues; 7 significantly upregulated, 1 downregulated; Supplementary Fig. S9E). This pertains to 10.81% affected genes for the DC-treated tissue (compared to that in the BC tissue), with 5.41% genes related to the apoptosis pathway, 2.70% genes to the antioxidant pathway, and 2.70% genes to the PI3K/Akt pathway (Fig. 3D).

3.2.3. CAF xxxx

The expression of 11 genes was altered in response to CAF treatment, compared to that in healthy tissue; 6 were significantly upregulated, while 5 were downregulated (Supplementary Fig. S9F). This pertains to 14.87% affected genes for the CAF-treated tissue, compared to that in the control, with 6.76% genes related to the apoptosis pathway, 8.11% to the antioxidant pathway, and no gene related to the PI3K/Akt pathway (Fig. 3E). The expression of 3 genes was significantly affected, compared to that in the BC tissues; 2 were significantly upregulated, while 1 was downregulated (Supplementary Fig. S9G). This pertains to 4% of affected genes for the CAF-treated tissue, compared to that in the BC tissue, with 2.67% genes related to the apoptosis pathway, 1.35% genes to the PI3K/Akt pathway, and no gene related to the antioxidant pathway (Fig. 3F).

3.2.4. CMF xxxx

The expression of 11 genes was altered in response to CMF treatment, compared to that in healthy tissue; 6 were significantly upregulated, while 5 were downregulated (Supplementary Fig. S9H). Similar to that for the CAF treatment, the expression levels of approximately 14.87% genes were altered in the CMF-treated tissue, compared to that in the healthy tissue, with 6.76% genes related to the apoptosis pathway, 8.11% genes to the antioxidant pathway, and no gene in the PI3K/Akt pathway (Fig. 3G). In addition, compared to that in the BC tissues, the expression of 2 genes was significantly altered; 1 was significantly upregulated, and 1 was downregulated (Supplementary Fig. S9I). This pertains to 2.7% affected genes for the CMF-treated tissue, compared to that in BC tissue, of which 1.35% genes were related to the apoptosis pathway, 1.35% genes to the PI3K/Akt pathway, and no gene in the antioxidant pathway (Fig. 3H).

3.2.5. BC tissues without treatment

BC tissues without any treatment exhibited significant changes in the expression of 9 genes, compared to that in the healthy tissue; 2 were significantly upregulated, while 7 were downregulated (Supplementary Fig. S9A). The expression levels of approximately 12.16% genes were altered in the BC tissue, compared to that in healthy tissue, with 4.05% genes related to the apoptosis pathway, 5.41% genes to the antioxidant pathway, and 2.70% genes to the PI3K/Akt pathway (Fig. 3I).

3.3. Summary of the effects of the treatments on gene expression

DC exhibited the greatest effect with respect to altering the expression of apoptosis-related genes, compared to that in the healthy tissue (13.33%), while the other treatments (TMX, CAF, and CMF) showed 11.11% change, each (Fig. 4A). When compared to the BC tissues, the DC-treated tissues exhibited the greatest change in the expression of apoptotic genes (8.70%), followed by the TMX- (6.52%), CAF- (4.35%), and CMF (2.17%)-treated tissues (Fig. 4B).

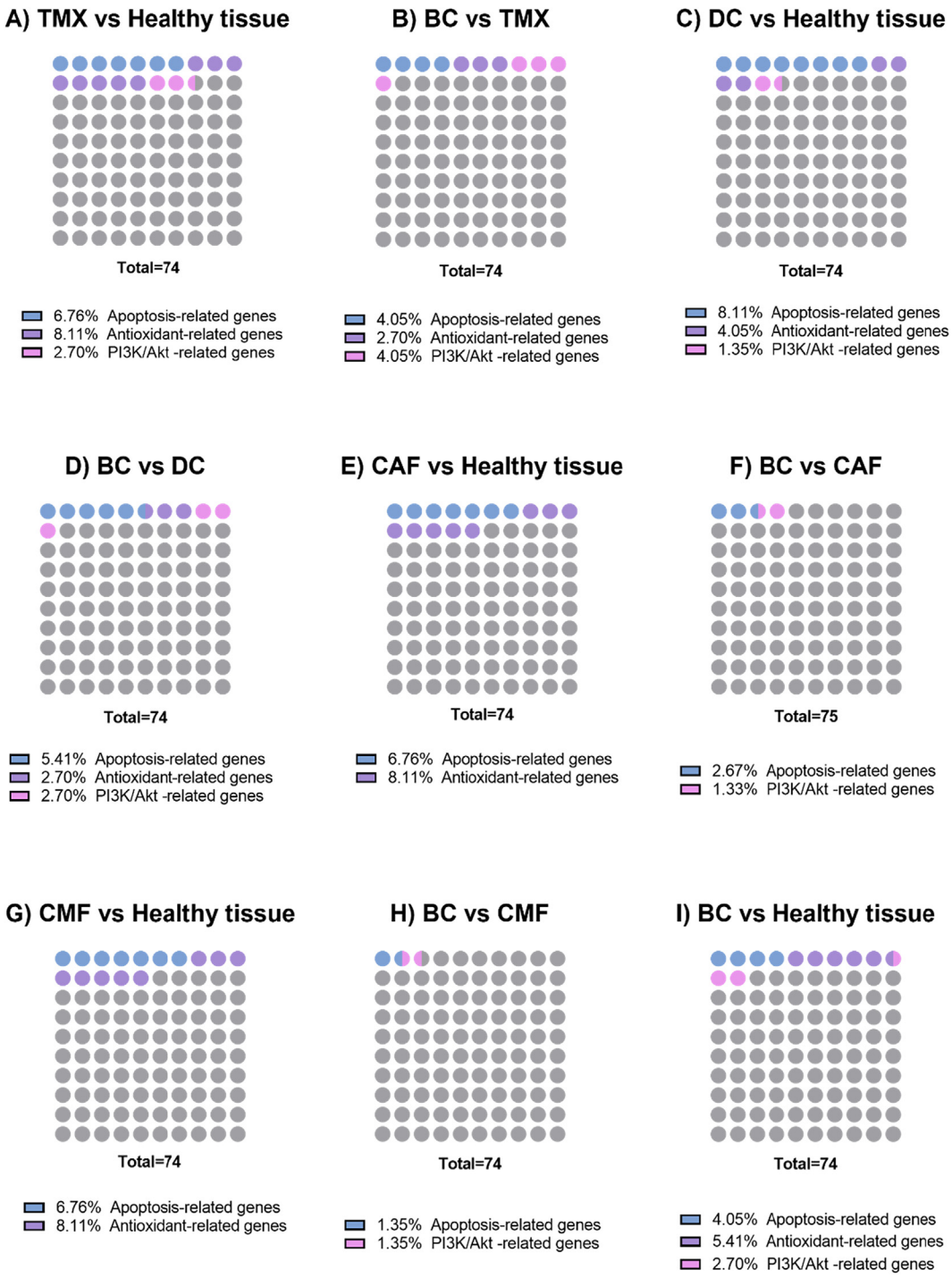

Fig. 3. The distribution of genes related to apoptosis, antioxidant, and PI3K/AKT pathways, following chemotherapy. Data for treatments with TMX, DC, CAF, or CMF, are expressed relative to those in healthy tissues or BC. BC, breast cancer tissues; TMX, tamoxifen; DC, combination of docetaxel and capecitabine; CAF, combination of cyclophosphamide, doxorubicin, and fluorouracil; CMF, combination of cyclophosphamide, methotrexate, and fluorouracil.

TMX, CAF, and CMF exhibited similar effects on the expression of genes in the antioxidant pathway (28.57% for each), while DC resulted in a lesser effect (14.29%) (Fig. 4C). TMX and DC exhibited similar effects on the expression of genes in the antioxidant pathway, compared to what is observed in BC tissues, (9.52%), while no changes were detected in response to CAF and CMF treatments (Fig. 4D). The expression of PI3K/Akt related genes was the most affected in TMX-treated tissues (25%), compared to that in the healthy tissue, while DC-treated tissues exhibited 12.50%, and no changes for CAF and CMF treatments (Fig. 4E). compared to BC, The expression of genes in the PI3K/Akt pathway exhibited the highest change (37.50%) in TMX-treated tissues, followed by DC-treated tissues (25%), and the CAF and CMF-treated tissues (12.50% each) (Fig. 4F).The genes affected in the three investigated

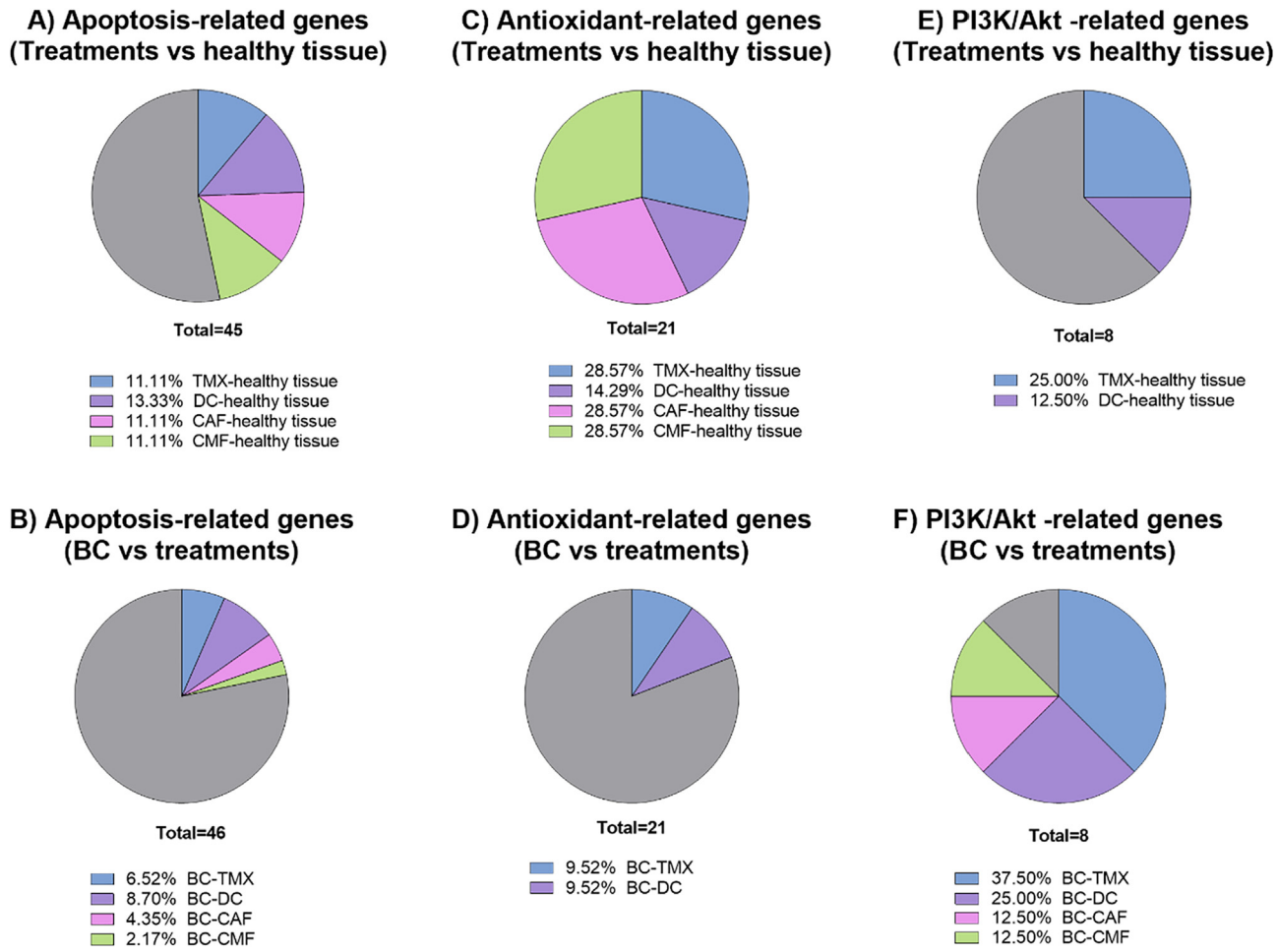

Fig. 4. Percentage of affected genes related to apoptosis, antioxidant pathway, and PI3K/AKT pathway following chemotherapy. Data are presented for treatments with TMX, DC, CAF, or CMF, and expressed relative to those in healthy tissues, or BC. A) Apoptosis-related genes (treatments vs healthy tissue), B) Apoptosis related genes (BC vs treatments), C) Antioxidant-related genes (treatments vs healthy tissue), D) Antioxidant-related genes (BC vs treatments), E) PI3K/Akt-related genes (treatments vs healthy tissue), and G) PI3K/Akt-related genes (BC vs treatments). BC, breast cancer tissues; TMX, tamoxifen; DC, combination of docetaxel and capecitabine; CAF, combination of cyclophosphamide, doxorubicin, and fluorouracil; CMF, combination of cyclophosphamide, methotrexate, and fluorouracil.

pathways are shown in Fig. 5, as a Venn Diagram of the genes either up- or downregulated for each treatment, relative to their expression in either healthy or BC tissue.

The differentially upregulated genes in each treatment vs healthy tissue are presented in (Fig. 5A). The expression of three genes *GSR, NOX4,* and *CASP3* was upregulated in all treated tissues, compared to that in the healthy tissue. The expression of the anti-apoptotic gene *BCL2A1* was upregulated in all treatments, except TMX (compared to that in healthy tissues). TMX and CMF upregulated the expression of *CASP6*, DC and CAF upregulated the expression of *CASP8*, and a combination of TMX and DC unregulated the expression of *CASP7*. The expression of *PMAIP1 (NOXA) was upregulated* in the TMX-, CAF-, and CMF- treated tissues, compared to that in healthy tissue, while it was downregulated in the BC tissue (Fig. 5A). Differentially downregulated genes in each treatment vs healthy tissue are presented in (Fig. 5B). The expression of three genes including two antioxidant genes, *GPX3* and *SOD3*, and the reactive species generator *XDH* gene was downregulated in tissues treated with TMX, CAF, and CMF (compared to that in healthy tissue). The expression of *MAOA* was significantly downregulated in CAF- and CMF-treated tissues (compared to that in the healthy tissue). The expression of the pro-apoptotic gene *BNIP3L* was downregulated in all the treatments, except TMX. The differentially upregulated genes in each treatment vs BC are presented in (Fig. 5C). The expression of only *ERBB2* was significantly downregulated in the treated tissues, compared to that in the BC tissue. The differentially downregulated genes in each treatment vs BC are presented in (Fig. 5D). The expression of *BCL2* was upregulated in the TMX- and DC- treated tissues (compared to that in the BC tissue).

4. Discussion

Chemotherapy has limitations, such as dosage toxicity, inconsistent clinical outcomes, and varying response rates. Most cancer patients share the same clinical characteristics; however, the responses to the treatments vary greatly, with even no response in some cases. The overuse of these drugs may result in adverse drug reactions or overdose. PM based on the genetic profile of the patient's cancer can evaluate the potential pharmacokinetics and pharmacodynamics of drug combinations and dosage, for the best and safest clinical outcomes (Vogenberg et al., 2010). However, not much information is available about the drug–gene pharmacogenomic interactions in BC. Therefore, this study aimed to highlight the interaction between established therapeutic drugs and the host's altered genetic profiles. In-depth analysis of five

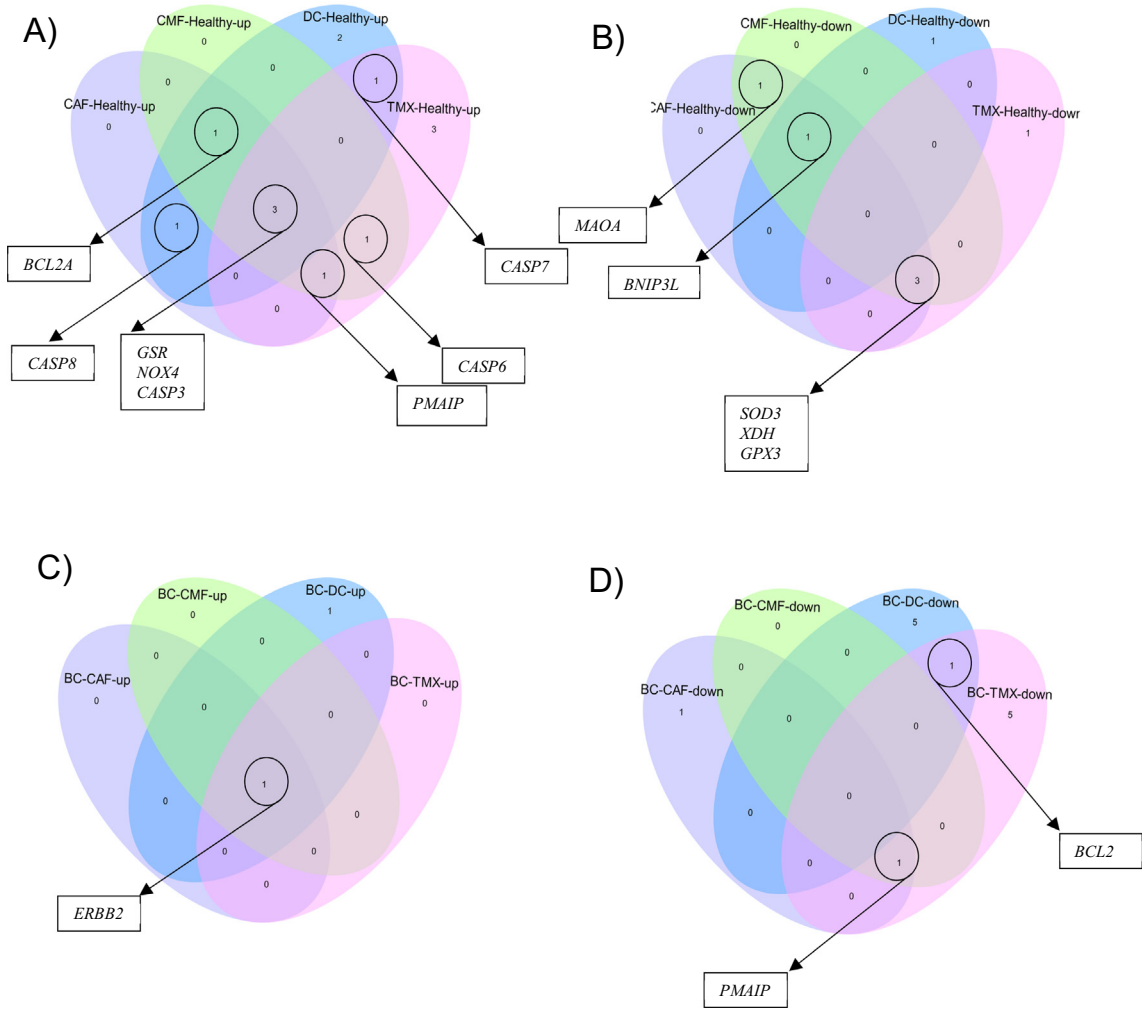

Fig. 5. Four-way Venn diagram illustrating the gene expression patterns (up- or downregulation) following chemotherapy. A) Differentially upregulated genes for each treatment vs healthy tissue. B) Differentially downregulated genes for each treatment vs healthy tissue. C) Differentially upregulated genes for each treatment vs BC. D) Differentially downregulated genes for each treatment vs BC. BC, breast cancer tissues; TMX, tamoxifen; DC, combination of docetaxel and capecitabine; CAF, combination of cyclophosphamide, doxorubicin, and fluorouracil; CMF, combination of cyclophosphamide, methotrexate, and fluorouracil.

published data sets that investigated the therapeutic effects in ER + ductal BC revealed over 74 genes related to the apoptosis pathway, oxidative stress-mediated antioxidant pathway, and PI3K/Akt pathway; their expression was compared between the healthy and BC tissues.

4.1. Apoptotic pathway alterations

Targeting apoptosis is the best nonsurgical treatment for many cancers. Therefore, novel apoptotic therapeutic strategies are considered for treating BC (Simstein et al., 2003). All the drugs examined in this study influenced the regulation of apoptotic genes, but the extent to which apoptosis is affected is not fully understood. Treatment with DC had the greatest effect on apoptotic gene expression. The differential expression of nine apoptosis-related genes also suggested that DC was the most effective chemotherapuetic for enhancing apoptosis. Docetaxel induces anti-tumor activity through the downregulation of intratumoral aromatase expression, representing an additional mechanism of docetaxel in hormonal therapy (Noguchi, 2006). DC was the only treatment to induce differential gene expression among the death receptor genes, as the expression of both *FAS* and *TNFRSF10B* were upregulated, when compared to that in BC tissue, and the expression of *TNFRSF10B* was upregulated, when compared to that in healthy tissue, suggesting that cellular apoptosis could occur quickly following DC treatment through *FAS*-mediated apoptosis. In primary prostate cells, docetaxel pre-treatment induces a three-fold upregulation in the expression of *FAS*, enhancing apoptosis (Symes et al., 2008). An *in vitro* study of the molecular pharmacology of capecitabine (Xeloda) in human colorectal tumor cells showed that Xeloda induces apoptosis through the *FAS*/*FASL* system (Ciccolini et al., 2002).

CASP3 was the only apoptotic gene to be commonly upregulated in all treatments; this is not surprising as overexpression of *CASP3* leads to apoptosis and BC development is correlated with *CASP3* downregulation (Végran et al., 2006; Nakopoulou et al., 2001; Huang et al., 2011a; Hu et al., 2007). The expression of *CASP3* reduces BC progression (Wu et al., 2012; Devarajan et al., 2002), though there are contradictory results (Hayes et al., 2001; Engels et al., 2013). Downregulating or knocking out *CASP3* in tumor cells resensitizes the tumor cells to treatments (Huang et al., 2011b). Treatment with TMX and DC upregulated *CASP7* expression. The relative expression of *CASP7* and ER alpha is dependent of the expression of these two proteins and is constantly upregulated in the various stages and grades of BC. Overexpression of *CASP7* is associated with good prognosis in BC patients (Chaudhary et al.,

2016). In addition, the overall survival rate in cancer patients corresponds to the expression level of *CASP* 1–8 (Wang et al., 2019). *CASP6* was upregulated by TMX and CMF, and *CASP8* was upregulated by DC and CAF. This suggests that these treatments may activate the extrinsic apoptosis pathway through upregulation of either *CASP6* or *CASP8*.

TMX, CAF, and CMF upregulated the expression of *PMAIP1* (*NOXA*) (compared to that in healthy tissue), while *PMAIP1* was downregulated in BC tissue with the same treatment. There is a positive correlation between the expression of *PMAIP1* and the overall survival rate in both primary and metastatic BC patients (Mamoor, 2020). The expression of the pro-apoptotic gene *BNIP3L* was downregulated in all treatments, except TMX, in this study. The expression of *BNIP3* is higher in BC tissue, compared to that in healthy breast tissue (Sowter et al., 2001) and in ductal carcinoma (*in situ*), associated with both high-grade and invasive tumors (Sowter et al., 2003). Overexpression of *BNIP3* is negatively associated with metastasis; while, silencing BNIP3 expression was found to result in increased tumor size and metastasis in an experimental *in vivo* model (Manka et al., 2005).

The expression of the anti-apoptotic gene, *BCL2A1* was upregulated in all the treatments, except in TMX (compared to that in the healthy tissues), while the expression of *BCL2* was upregulated in TMX- and DC- treatment (compared to that in the BC tissue). BCL2A1 translocates from the cytoplasm to the nucleus during the initiation of apoptosis; however, its exact function in healthy and tumor cells remains unknown (Hind et al., 2015; Vogler, 2012). In cancer cells, the expression of *BCL2A1* is often upregulated and it plays a role in the development of resistance against therapeutic agents that induce apoptosis. In healthy breast tissue, *BCL2* is overexpressed in response to estrogen (Leek et al., 1994; Sabourin et al., 1994). The expression of *BCL2* is associated with better outcomes, such as smaller tumors and delayed proliferation (Silvestrini et al., 1994). The expression of *BCL2* correlates favorably with prognosis, specifically in patients who undergo hormone therapy (Callagy et al., 2008; Yang et al., 2013; Silvestrini et al., 1994; Van Slooten et al., 1996; Veronese et al., 1998; Hellemans et al., 1995; Lipponen et al., 1995; Silvestrini et al., 1996; Elledge et al., 1997; Callagy et al., 2006).

4.2. Oxidative stress mediates antioxidant pathway alterations

Oxidative stress has a multifactorial effect in response to treatment cycles. The effects on BC patients following the initial changes in chemo-induced oxidative stress is unclear (Kok et al., 2012). Oxidative stress facilitates TMX-induced killing of BC cells, therefore, enhancing the drug's effectiveness (Bekele et al., 2016). We investigated the effect of the treatments on the expression of genes in the ROS pathways to elucidate their roles in the antioxidant gene defense cascade. The percentage of genes differentially expressed was similar among the three treatments, TMX, CAF, and CMF; but not DC, which exhibited a weaker effect. Only TMX and DC-treated tissues exhibited differential expression in the oxidative stress genes, when compared to that in BC. The expression of *NOX4* was markedly upregulated in all treatments, compared to that in the healthy control. NOX4 regulates cell growth and proliferation in the liver, affecting both cellular homeostasis and carcinogenesis (Crosas-Molist et al., 2014). In animal models of hepatocellular carcinoma, the knockdown of *NOX4* induces tumorigenesis, forming an early tumor (Crosas-Molist et al., 2017). In normal mammary epithelial cells, upregulation of *NOX4* leads to cell senescence, resistance to apoptotic cell death, and transformation into cancer cells. *NOX4* upregulation is proposed to generate ROS in the mitochondria (Graham et al., 2010). Earlier studies evaluating the expression of *NOX4* used cell lines; and therefore, elucidating the mechanism *in vivo* in breast cancer patients is important.

The expression of *XDH* was significantly downregulated in tissues treated with TMX, CAF, and CMF, compared to that in healthy tissue. Overexpression of *XDH* induces ROS generation and stimulates DNA damage, tumorigenesis, and metastasis (Battelli et al., 2016; Sabharwal and Schumacker, 2014; Matsui et al., 2000; Romagnoli et al., 2010). The downregulation of *XDH* is a predictor of poor prognosis in many types of cancers, including BC (Battelli et al., 2016; Linder et al., 2005; Linder et al., 2012; Kim et al., 2011). The relationship between *XDH* downregulation and the development and progression of several cancers, is still unknown.

The expression of *MAOA* was significantly downregulated in tissues treated with CAF and CMF, compared to that in healthy tissue. In BC, the expression of *MAOA* differs according to the molecular subtype. In luminal-type BC, the expression of *MAOA* is upregulated, and appears to be higher in chemically induced BC in animal models (Lizcano et al., 1991; Lizcano et al., 1990). Activation of ER receptors upregulated the expression of *MAOA* (Ren et al., 2011). The expression of *MAOA* is downregulated in almost all cancers, as observed using a DNA array (Sun et al., 2017). The expression of *MAOA* is downregulated in human BC and human basal-like BC, when compared to that in noncancerous cells (Ren et al., 2011). The expression of *MAOA* induces tumor metastasis and is associated with the initiation of BC (Gwynne et al., 2019).

The expression of *GSR* was upregulated in all the treated tissues. This gene encodes glutathione reductase (GSR) that plays a role in the establishment of cellular antioxidant defenses (Couto et al., 2016). Glutathione (GSH) is an important antioxidant vital for cell signaling and maintaining the redox balance (Circu and Aw, 2012). GSH deficiency affects the clinical outcomes of cancer patients (Bansal and Simon, 2018). Treatment resistance is associated with elevated expression of *GSR* and oxidative stress-induced damage (Kim et al., 2010). Downregulation of genes related to glutathione homeostasis results in increased susceptibility of cancer cells to antioxidants, particularly thioridazine (Yan et al., 2019). Therefore, GSH can have protective and pathogenic effects. Targeting *GSR* enhances the efficacy of drugs and reduces drug resistance (Balendiran et al., 2004; Majumder et al., 2020). However, in primary human tumors, the effect of modulating the expression of this gene needs evaluation.

The antioxidant genes, *GPX3* and *SOD3,* were downregulated in tissues treated with TMX, CAF, and CMF. *GPX3* is a tumor suppressor in BC and is therefore downregulated in normal cases (Lou et al., 2020). *GPX3* targets the Wnt5a/JNK pathway in cancer cells and inhibits cell migration and invasiveness (Lou et al., 2020). In lung cancer, *GPX3* is an oncogene that acts as a tumor suppressor (An et al., 2018). Silencing of *GPX3* induces metastasis (Zhao et al., 2015). Plasma *GPX3* inhibits colitis-associated colorectal cancer (Barrett et al., 2013). The role of *GPX3* in BC needs further exploration.

SOD3 encodes an enzyme that hydrolyses O_2^- and forms OH (Carlsson et al., 1995). The role of *SOD3* in cancer biology is majorly unknown; however, the downregulation of *SOD3* is associated with poor prognosis of cancer (Chaiswing et al., 2008; Liu et al., 2016). The expression of *SOD3* is regulated by the NRF2 pathway and it may play an important role in inducing antioxidant defense against oxidative stress; *SOD3* inhibits BC development in presence of estrogen (Singh and Bhat, 2012).

4.3. PI3K/Akt signaling pathway alterations

The PI3K/Akt pathway plays an important role in the regulation of cellular processes, including cell proliferation. It is associated with tumor progression and is commonly activated in human can-

cers. Therefore, novel PI3K/Akt pathway inhibitors are one of the most effective tumor therapeutics (Shi et al., 2019).

ERBB2 (*HER2*) activates the PI3K/Akt pathway through direct or indirect mechanisms (Ruiz-Saenz et al., 2018); *ERBB2* upregulation is associated with aggressive tumors (Hung et al., 1986). In this study, almost all the drugs modulated the expression of genes involved in this pathway at levels similar to those in healthy tissue. *ERBB2* expression was significantly downregulated in the treated tissues, compared to that in BC tissues. *ERBB2* is overexpressed in approximately 30% of the BCs (Slamon et al., 1989); and this upregulation is associated with markedly lower survival rates, frequent relapses, or both. In addition, overexpression of ERBB2 inhibits apoptotic stimuli (Xia et al., 2006), and increases metastasis in BC (Tan et al., 1997; Moody et al., 2002; Holbro et al., 2003). This is in line with this study's findings that chemotherapies induce upregulation of the genes encoding proteins involved in the apoptotic pathway. Therefore, *ERBB2* inhibition is a valuable therapeutic target in BC (Shawver et al., 2002).

This study had some limitations. First, the datasets did not provide clear information regarding survival (outcomes), relapse, drug resistance, or prognosis. In addition, information regarding the dosages of some drugs and the treatment durations was missing. Lastly, there was no mention of factors that might influence drug metabolism and overall effects; information regarding these aspects could have enabled us to reach a clearer conclusion.

5. Conclusion

BC treatment is complicated and involves heterogeneous factors. Chemotherapy is used to treat BC; however, it is not effective in the majority of patients. BC patients who respond poorly to chemotherapy require analysis of specific molecular biomarkers to predict their treatment response and to limit the toxic side effects. Despite the availability of information pertaining to signaling pathways associated with apoptosis, oxidative stress/antioxidant and PI3K/Akt, more information is needed for their selective manipulation. Understanding the underlying genetic mechanism will offer attractive new opportunities for treating BC. We aimed to improve the current knowledge regarding the relationship between these genes, known chemotherapeutics, and the clinical response. Our study expands the selection of anticancer agents and personalized treatment options for BC patients. Our findings suggest the possibility of using *GSR, NOX4, CASP3*, and *ERBB2* as potential biomarkers for predicting the therapeutic response in primary ER+ ductal breast carcinoma.

Authors contributions

YA, and SMA conception and design of the study, acquisition, analysis, and interpretation of data, drafting, and revising the article, AA, AAA, and SA acquisition and analysis of data. All authors had final approval of the submitted manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declared none.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2021.04.027.

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