Network Pharmacology-Based Dissection of the Comprehensive Molecular Mechanisms of the Herbal Prescription FDY003 Against Estrogen Receptor-Positive Breast Cancer

Ho-Sung Lee¹², In-Hee Lee¹, Kyungrae Kang², Sang-In Park³, Minho Jung², Seung Gu Yang⁴, Tae-Wook Kwon², and Dae-Yeon Lee¹²

Abstract
Estrogen receptor-positive breast cancer (ERPBC) is the commonest subtype of breast cancer, with a high prevalence, incidence, and mortality. Herbal drugs are increasingly being used to treat ERPBC, although their mechanisms of action are not fully understood. Therefore, in this study, we aimed to analyze the therapeutic properties of FDY003, a herbal anti-ERPBC prescription, using a network pharmacology approach. FDY003 decreased the viability of human ERPBC cells and sensitized them to tamoxifen, an endocrine drug that is widely used in the treatment of ERPBC. The network pharmacology analysis revealed 18 pharmacologically active components in FDY003 that may interact with and regulate 66 therapeutic targets. The enriched gene ontology terms for the FDY003 targets were associated with the modulation of cell survival and death, cell proliferation and growth arrest, and estrogen-associated cellular processes. Analysis of the pathway enrichment of the targets showed that FDY003 may target a variety of ERPBC-associated pathways, including the PIK3-Akt, focal adhesion, MAPK, and estrogen pathways. Overall, these data provide a comprehensive mechanistic insight into the anti-ERPBC activity of FDY003.

Keywords
Herbal drugs, network pharmacology, estrogen receptor-positive breast cancer, anticancer agents, molecular mechanisms

Received: March 26th, 2021; Accepted: August 17th, 2021.

Introduction
Breast cancer (BC) is a potentially fatal malignancy, with 2.3 million new cases and 0.7 million deaths reported globally every year.¹ BCs can be divided into three types: estrogen receptor-positive BC (ERPBC), human epidermal growth factor receptor 2 (HER2)-amplified BC, and triple-negative BC; ERPBC is the most commonly occurring type.² Selective estrogen receptor modulators, aromatase inhibitors, and selective estrogen receptor down-regulators are commonly used for the treatment of ERPBC.³ These endocrine therapies exhibit low clinical efficacy due to the development of drug resistance, and cause adverse events, including hot flashes, osteoporosis, arthralgia, and cardiovascular symptoms.⁴ To overcome these challenges, herbal drugs that have substantial efficacy and fewer side effects are now being prescribed for the treatment of cancer.⁵⁻⁶ Studies have demonstrated the effects of herbal drugs for improving survival and clinical outcomes, enhancing the treatment rate, reducing adverse effects, and augmenting the health status and quality of life of cancer patients.⁷⁻⁸ FDY003 is a herbal anticancer prescription containing Lonicera japonica Thunberg (LjT), Artemisia capillaris Thunberg (AcT), and Cordyceps militaris (Cm).⁹⁻¹¹ FDY003 can suppress cell proliferation and induce apoptosis in various cancers by modulating important signaling pathways and regulators that are involved in the coordination of cellular proliferation, growth, survival, and apoptosis.³⁻¹¹ However, the systemic role of FDY003 in the treatment of ERPBC has not been investigated.

¹The Fore, Seoul, Republic of Korea
²Forest Hospital, Seoul, Republic of Korea
³Forestheal Hospital, Seoul, Republic of Korea
⁴Kyunghee Naro Hospital, Seongnam, Republic of Korea

Corresponding Authors:
Dae-Yeon Lee, Forest Hospital, 129 Ogeum-ro, Songpa-gu, Seoul 05549. Republic of Korea. E-mail: foresthrnd@gmail.com
Ho-Sung Lee, Forest Hospital, 129 Ogeum-ro, Songpa-gu, Seoul 05549. Republic of Korea. E-mail: forehslee@gmail.com

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access page (https://us.sagepub.com/en-us/nam/open-access-at-sage).
The complex systematic multicomound-multitarget pharmacological characteristics of herbal drugs present a major challenge in the investigation of their therapeutic mechanisms and effects. To overcome this hurdle, the network pharmacology approach has been applied to facilitate the study of the comprehensive systemic mechanisms of herbal drugs. The therapeutic activity of polypharmacological herbal drugs is exerted through the interactions of their constituent phytochemicals with multiple biological components. Therefore, the goal of network pharmacology is to identify the bioactive components of herbal drugs and the disease-associated genes and proteins that they target. Thereafter, the multicomound-multitarget features of the herbal drugs are analyzed by exploring the mechanisms underlying the interactions between the compounds and their biological targets, as well as the biomedical functions and characteristics of the targets. In this study, we assessed the therapeutic effects of FDY003 against ERPBC and employed network pharmacology to explore its comprehensive anticancer mechanisms.

Materials and Methods

Cell Culture

MCF-7 human ERPBC cells were obtained from the Korean Cell Line Bank (Seoul, Korea). The cells were cultured in Dulbecco’s modified Eagle’s medium (WELGENE Inc., Daegu, Korea) with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (Thermo Fisher Scientific Inc., Waltham, MA, USA) and incubated at 37 °C in a humidified atmosphere containing 5% CO₂.

Preparation of FDY003

The herbal medicines LjT, Act, and Cm were purchased from Hanpure Pharmaceuticals (Pocheon, Korea). Dried LjT (4.16 g), Act (6.25 g), and Cm (6.25 g) were ground and immersed in 70% ethanol (500 mL). An extract was prepared by refluxing the mixture at 80 °C for 3 h. The extract was then filtered, purified with 80% and 90% ethanol successively, and lyophilized at −80 °C. The samples were stored at −20 °C and dissolved in distilled water for use in further experiments.

Cell Viability Assay

Cell viability was assessed using the WST-1 assay. Briefly, 1.0 × 10⁴ cells were seeded in a 96-well plate and treated with the indicated doses of FDY003 and tamoxifen (Sigma-Aldrich, St. Louis, MO, USA) for 72 h. WST-1 solution (Daecil Lab Service Co., Ltd, Seoul, Korea) was added to each well, and the cells were incubated for an additional 2 h. The absorbance was measured at 450 nm using an xMark microplate absorbance spectrophotometer (Bio-Rad, Hercules, CA, USA).

Bioactive Phytochemical Compounds of FDY003

A list of the chemical components of FDY003, and their absorption, distribution, metabolism, and excretion profiles were obtained from the Traditional Chinese Medicine Systems Pharmacology, Anticancer Herbs Database of Systems Pharmacology, Anticancer Herbs Database of Systems Pharmacology, Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine, and Traditional Chinese Medicine Integrated Database. Next, we assessed the parameters that are widely used in network pharmacology studies to determine which components were most likely to be pharmacologically active. Caco-2 permeability is an index for assessing the intestinal permeability of a compound by using a Caco-2 human intestinal cell model. A Caco-2 permeability ≥0.4 is indicative of good in vivo intestinal permeability. Drug-likeness is an index of the potential of a compound to be used as a drug, based on its structural and physicochemical features, molecular descriptors, and Tanimoto coefficient. The mean drug-likeness score of approved drugs is reported to be 0.18; therefore, compounds with a score ≥0.18 were considered potentially druggable. Oral bioavailability describes a compound’s ability to reach the target site (tissues and organs) after oral administration. A compound with oral bioavailability ≥30% is considered to be effectively absorbed and distributed in the body. Therefore, phytochemical compounds with Caco-2 permeability of −0.4 or higher, drug-likeness ≥0.18, and oral bioavailability ≥30% were considered bioactive.

Targets of Bioactive Phytochemical Compounds of FDY003

The canonical simplified molecular input line entry specification (SMILES) notations of the bioactive phytochemical components of FDY003 were obtained from the PubChem database. The SMILES notations were imported into SwissTargetPrediction, PharmMapper, Search Tool for Interactions of Chemicals, and Similarity Ensemble Approach to determine the biological target of each compound in Homo sapiens by investigating the chemical-protein interactions. The genes and proteins associated with ERPBC in humans were retrieved from a previous network pharmacology study and from other databases (DisGeNET, Therapeutic Target Database, Online Mendelian Inheritance in Man, GeneCards, and DrugBank) using the search term “Estrogen receptor-positive breast cancer”.

Herbal Prescription-Associated Networks

Pharmacological networks were constructed to facilitate further data analysis. The herbal medicine-bioactive compound-target (H-C-T) network mapped the links among herbal medicines, their bioactive phytochemical constituents, and their ERPBC-related therapeutic targets. The H-C-T-P network outlined the connections between the targets in the H-C-T network, and the pathways in which they are involved. The
protein–protein interaction (PPI) network summarized paired interactions between ERPBC-associated targets retrieved from the STRING database. All networks were constructed, visualized, and analyzed using the Cytoscape software. The herbal prescription-associated network is composed of nodes (herbal and bioactive phytochemical constituents, targets, and pathways) and links (or edges) that represent the interactions between them. The degree indicates the number of links that the nodes contain.

**Survival Analysis**

To assess the clinical significance and therapeutic importance of the FDY003 targets, we conducted a survival analysis. The correlation between the survival rates of patients with ERPBC and the FDY003 targets was analyzed with the Kaplan–Meier Plotter using auto-selected best cutoffs and the log-rank test. Differences with \( P < 0.05 \) were considered statistically significant.

**Functional Enrichment Analysis**

To explore the key molecular and pathway mechanisms of action of FDY003 against ERPBC, we conducted a functional enrichment analysis for the FDY003 targets. Therefore, we imported the targets into g:Profiler, a useful tool for the functional investigation of genes of interest, and obtained the gene ontology terms and pathways wherein they are significantly enriched \( (P < 0.05) \).

**Molecular Docking Assessment**

The structural information of phytochemical compounds and targets was retrieved from PubChem and RCSB Protein Data Bank, respectively. These data were imported into Autodock Vina and the molecular docking scores for interactions between the phytochemical compounds and targets were calculated. A docking score of \(-5.0\) or less was considered indicative of strong binding.

**Results**

**Anticancer Properties of FDY003 Against BC**

To assess the anticancer effects of FDY003 on ERPBC, MCF-7 cells were treated with FDY003 in the presence or absence of tamoxifen, an endocrine drug that is clinically used for the treatment of ERPBC, and the cellular responses were evaluated. FDY003 was found to decrease the viability of MCF-7 cells and enhance the anti-proliferative effect of tamoxifen (Supplementary Figure S1), indicating that FDY003 may possess anti-ERPBC activity.

**Identification of Bioactive Phytochemical Compounds of FDY003 and Their Targets**

We assessed the pharmacokinetic properties of the phytochemical components of FDY003 (Supplementary Table S1), and shortlisted potentially bioactive compounds that satisfied the set criteria (Caco-2 permeability \( \geq -0.4 \) or higher, and drug-likeness \( \geq 0.18 \), oral bioavailability \( \geq 30\% \)), as previously described. Therefore, 18 bioactive compounds were determined for FDY003 (Supplementary Table S2). Then, the human targets of FDY003 were screened based on the molecular structures of the bioactive compounds (see Materials and Methods). In total, 66 ERPBC-associated and 130 non-ERPBC-related targets were determined for FDY003 (Supplementary Table S3).

**Network Pharmacological Mechanisms of FDY003 Against ERPBC**

The comprehensive data associated with the bioactive phytochemical compounds and targets of FDY003 were integrated into an H-C-T network (Figure 1). The network was composed of 87 nodes (3 herbal medicines, 18 bioactive phytochemical compounds, and 66 ERPBC-associated targets) and 134 edges (Figure 1 and Supplementary Table S3). The bioactive compounds with the highest number of targets were kaempferol, luteolin, and quercetin (Figure 2 and Supplementary Table S3), suggesting that these agents may be the major pharmacological components. Moreover, 83% of the targets (55 out of 66 nodes) interacted with two or more compounds (Figure 2), implying the polypharmacological features of FDY003.

Mechanistic investigation of the complex interactions between target genes and proteins is essential for unraveling the therapeutic properties of a drug. Therefore, we constructed a PPI network (65 nodes and 332 links) using the ERPBC-associated targets as nodes (Figure 2). Then, we explored the hubs within the PPI network, nodes that possess relatively many links and function as crucial biological regulators and also as promising drug targets. As previously described, hubs were defined as nodes where the number of links was greater than or equal to twice the mean node degree of the network.

**Results**

**Anticancer Properties of FDY003 Against BC**

To assess the anticancer effects of FDY003 on ERPBC, MCF-7 cells were treated with FDY003 in the presence or absence of tamoxifen, an endocrine drug that is clinically used for the treatment of ERPBC, and the cellular responses were evaluated. FDY003 was found to decrease the viability of MCF-7 cells and enhance the anti-proliferative effect of tamoxifen (Supplementary Figure S1), indicating that FDY003 may possess anti-ERPBC activity.
Figure 1. The herbal medicine-bioactive phytochemical compound-target network of FDY003. Green nodes, herbal medicines; red nodes, bioactive phytochemical compounds; blue nodes, estrogen receptor-positive breast cancer-related targets.

Figure 2. The protein-protein interaction network for estrogen receptor-positive breast cancer-related targets of FDY003. Pink nodes, hub estrogen receptor-positive breast cancer-related targets.
Functional Analysis of the Pharmacological Properties of FDY003

To dissect the key mechanisms of action of FDY003 against ERPBC, we conducted a functional enrichment analysis. Gene ontology enrichment analysis demonstrated that the targets of FDY003 participate in the regulation of cell survival and death, cell proliferation and growth arrest, and estrogen-associated cellular processes (Supplementary Figure S1). Furthermore, pathway enrichment analysis revealed that the targets are major constituents of the pathways associated with ERPBC pathology (Figure 4 and Supplementary Figure S2), suggesting the key signaling mechanisms of FDY003. The adenosine monophosphate (AMP)-activated protein kinase (AMPK) pathway exerts tumor-suppressing effects by inducing cell cycle arrest and apoptosis, and inhibits the growth and survival of ERPBC cells. The chemokine, erythroblastic leukemia viral oncogene homolog (ErbB), focal adhesion, mitogen-activated protein kinase (MAPK), PI3K-Akt, and Ras pathways play important roles in the initiation and progression of ERPBC by controlling diverse tumorigenic behaviors in cells (e.g., angiogenesis, cancer stemness, metastasis, migration, invasion, proliferation, survival, and tumor-initiating capacity). The dysregulated synthesis and secretion of estrogen and its downstream activity are the primary drivers of tumorigenesis and progression of ERPBC; pharmacological modulation of estrogen and the pathways in which it is involved is a key strategy in anti-ERPBC therapeutics. The hypoxia-inducible factor (HIF)-1 pathway coordinates the self-renewal potential, cancer stemness, proliferation, and metastasis of ERPBC cells. Furthermore, insulin resistance may be associated with the

Figure 3. Survival analysis of estrogen receptor-positive breast cancer-associated targets of FDY003. Kaplan-Meier curves for the survival of patients with estrogen receptor-positive breast cancer according to the expression levels of the indicated estrogen receptor-positive breast cancer-associated targets of FDY003.

Lee et al.
incidence, recurrence, and survival rates of BC patients. The loss of p53 pathway function remains one of the most important events in the pathophysiology of ERPBC and is associated with disease aggressiveness and survival outcomes in patients with BC. The activity of the PD-1/PD-L1 pathway is an indicator of survival in BC patients, and blocking this pathway may strengthen anti-tumor immune function. The prolactin pathway functions as a survival and migration factor for ERPBC cells and is a risk factor for BC; therefore, inhibition of this pathway can reduce the cancerous ability of ERPBC cells and make them more susceptible to anticancer drugs. The tumor necrosis factor (TNF) pathway modulates pro-tumorigenic inflammation, and its activity is associated with carcinogenesis, malignant tumor development, metastasis, induction of therapeutic resistance, and prognosis of ERPBC. The VEGF pathway contributes to ERPBC progression by stimulating metastasis, cell survival, and angiogenesis in ERPBC tumors. The misregulation of important cellular processes, including apoptosis, cell cycle processes, and senescence is associated with uncontrolled growth, proliferation, and survival of ERPBC cells. The development of resistance against anticancer agents, such as endocrine therapeutics, platinum-based cytotoxic drugs, and EGFR inhibitors, is largely responsible for the failure of ERPBC treatment.

Overall, these results reveal the key molecular mechanisms and pathways underlying the anti-ERPBC activity of FDY003.

Molecular Docking Assessment of Bioactive Compounds and the Targets of FDY003

Molecular docking analysis is a reliable and convenient method to investigate and predict the intermolecular interactions between drug molecules and targets by evaluating their potential binding affinities. To assess the binding capacity of the bioactive components of FDY003 with their biological targets, we conducted molecular docking studies (see Materials and Methods). The docking scores of the bioactive compounds with their hub targets were found to be −5.0 or less (Figure 5), indicating the binding potential of the compound-target pairs. With regard to the binding interaction sites of the compound-target pairs, kaempferol mainly interacted with the amino acid residues Ala748, Met745, and Trp751 in androgen receptor (AR); amino acid residues Ala722, Arg836, Arg858, Arg860, Glu758, Gly724, Leu747, and Phe723 in EGFR; and amino acid residues Arg394, Leu387, and Phe404 in ESR1 (Figure 5). Luteolin may bind to MAPK8 by interacting with its amino acid residues such as Ala113, Asp112, Asp169, Leu178, and Val404 in ESR1 (Figure 5). Quercetin primarily formed interactions with amino acid residues Glu85, Ile84, and Lys875 in EGFR; amino acid residues Ala53, Gly33, Ile32, Ile39, Lys55, Ser34, and Val40 in MAPK8; and amino acid residues Gln165 and Gln167 in TP53 (Figure 5).
Discussion

There has been a growing interest in the use of medicinal herbs for the treatment of ERPBC, however, their polypharmacological mechanisms have not yet been explored from a network perspective. In this study, we dissected the multicomound-multitarget anti-ERPBC mechanism of the herbal prescription, FDY003. FDY003 decreased the...
viability of human ERPBC cells and sensitized them to endocrine therapy. Evaluation of the network pharmacology revealed that FDY003 contains 18 pharmacologically active compounds that may interact with and regulate 66 potential therapeutic targets. The enriched gene ontology terms of the FDY003 targets were found to be associated with the modulation of cell survival and death, cell proliferation and growth arrest, and estrogen-associated cellular processes. Pathway enrichment investigation revealed the crucial ERPBC-associated signaling pathways that may be targeted by FDY003: the PI3K-Akt, focal adhesion, MAPK, and estrogen pathways. Overall, the network pharmacological analysis revealed the molecular and signaling mechanisms underlying the anti-ERPBC activity of the herbal prescription.

The crucial ERPBC-associated hub targets of FDY003 are the key mediators that coordinate the pathological mechanisms involved in ERPBC and serve as potential therapeutic targets. The abnormal activation and deactivation of AKT1 (encoded by AKT1) and epidermal growth factor receptor (EGFR; encoded by EGFR) induce cancerous cellular behavior in ERPBC cells (eg, survival, metastasis, proliferation, cancer stemness, invasion, and migration); their expression, activity, and mutation status are prognostic predictors of therapeutic sensitivity and survival in patients with BC.\(^{65,107-120}\) Furthermore, these kinases are implicated in the occurrence of therapeutic resistance to anti-ERPBC treatment, and thus can be targeted to overcome this resistance.\(^{65,109-111,117,121}\) The activation of AR (encoded by AR) weakens the invasion, proliferation, migration, and tumor-initiating properties of ERPBC cells,\(^{122}\) and the expression of AR correlated with reduced mortality and improved prognostic outcome of ERPBC patients.\(^{123,124}\) Genetic alterations in and over-activation of estrogen receptor α (ERα; encoded by ESR1) are the key driving forces for the tumorigenesis and progression of ERPBC, and ERα is the main therapeutic target for endocrine therapies.\(^{125}\) In addition, ESR1 expression and mutation status are the determinants of endocrine resistance and clinical prognosis of ERPBC patients.\(^{126-130}\) Interleukin-6 (IL-6; encoded by IL6) is a cytokine that is involved in the growth, malignancy, invasiveness, and metastatic capability of ERPBC tumors, and its expression is highly upregulated in cancer patients.\(^{131-135}\) The expression, activity, and genetic variants of IL-6 are further associated with the risk of cancer incidence, survival, recurrence, and the development of therapy-induced toxicity in patients with BC.\(^{136-139}\) c-Jun N-Terminal Protein Kinase 1 (JNK1; encoded by MAPK8) regulates the survival, apoptosis, metastasis, and inflammation of ERPBC cells, and it also mediates the pharmacological effects of anticancer drugs.\(^{140-145}\) Malfunction and loss-of-function polymorphisms of TP53 induce the development and progression of ERPBC, and the expression and mutation status of this gene are correlated with the survival and prognostic outcomes of BC patients.\(^{77,140-154}\) Vascular endothelial growth factor (VEGF)-A (encoded by VEGFA) may contribute to the metastasis, angiogenesis, growth, and drug resistance of ERPBC cells,\(^{155-157}\) and its expression level and polymorphisms may act as genetic markers for the risk, progression and recurrence, clinical outcomes for endocrine and antiangiogenic therapies, and survival of patients.\(^{158-162}\) These results provide a pharmacological basis for the anti-ERPBC mechanisms of and further suggest the need for an in-depth investigation of FDY003’s therapeutic effects on multiple malignant cellular behaviors, including invasion, migration, metastasis, anoikis resistance, cancer stemness, and angiogenesis in ERPBC cells as investigated from the functional enrichment analysis (Supplementary Figure 2).

The components of FDY003 have previously been shown to exhibit anti-ERPBC activity. AcT has been found to reduce the proliferation and survival capacity of ERPBC cells.\(^{163}\) Cm represses the migration, survival, and growth of ERPBC cells by activating caspase-dependent mitochondrial signaling.\(^{164,165}\) Capillarisin exhibits anticancer effects by blocking the invasion and proliferation of ERPBC cells.\(^{166}\) The inhibitory effects of cordycepin on the invasion, survival, and proliferation of ERPBC cells are mediated by the caspase and MAPK pathways.\(^{167,168}\) Cordycepin can enhance the anticancer effects of radiotherapy on ERPBC cells.\(^{170}\) Genkwanin targets PI3K-Akt signaling to exhibit its anti-growth and pro-apoptotic activities in ERPBC cells.\(^{171,172}\) Isohamnetin inhibits cell cycle progression and proliferation of ERPBC cells.\(^{173}\) β-Sitosterol promotes the death of ERPBC cells and improves the efficacy of endocrine therapy in the treatment of ERPBC.\(^{175}\) Quercetin, luteolin, and kaempferol induce cell-cycle arrest, growth arrest, and cell death, while weakening proliferation, angiogenesis, metastasis, tumor-initiating capacity, invasion, and migration by targeting various signaling pathways associated with key ERPBC pathomechanisms.\(^{176-190}\) In addition, these compounds function as chemosensitizers that make ERPBC cells more susceptible to other anticancer drugs.\(^{191-193}\) Furthermore, the compound-target interaction relationship that was identified from the network pharmacological analysis (Supplementary Table S3) was previously reported. Cordycepin and quercetin increased the activity of caspase-9 (encoded by CASP9) to induce apoptosis of ERPBC cells.\(^{168,177}\) Kaempferol inactivates glucose transporter 1 (GLUT1; encoded by SLC2A1) and ERα, which suppresses the growth and survival capacity of ERPBC cells.\(^{176,180}\) Luteolin and quercetin target matrix metalloproteinase 2 (MMP2; encoded by MMP2) and MMP9 (encoded by MMP9) and exhibit anti-invasive effects on ERPBC cells.\(^{181,194}\) Quercetin, a pharmacological flavonoid,\(^{195}\) possesses anti-angiogenic ability against ERPBC cells by targeting c-Jun (encoded by JUN), VEGF-A, and VEGF receptor 2 (VEGFR-2; encoded by KDR)\(^{192,196,197}\) The suppression of cyclooxygenase-2 (COX-2; encoded by PTGS2) by quercetin contributes to the reduction of the invasiveness of ERPBC cells.\(^{198}\) Moreover, this active compound can downregulate AKT1, EGFR, and HIF-1α (encoded by HIF1A), while upregulating p53 (encoded by TP53) to inhibit the proliferation, chemoresistance, survival, cancer stemness, and invasion of
ERPBC cells. Further experimental studies are warranted to verify the physicochemical interactions and biological regulations between the active compounds and their targets for the investigation of the precise mechanisms of the herbal prescription.

In conclusion, this network pharmacological study gave us a comprehensive view of the molecular and signaling mechanisms involved in the anti-ERPBC activity of the herbal prescription, FDY003. The overall analysis results provide a systematic insight that can advance our understanding of the multicomponent-multitarget properties of herbal drugs as anticancer agents. To broaden the potential pharmacological applications of FDY003, the effects of FDY003 must be studied further, and the agent’s activity must be evaluated in combination with other anticancer agents (eg, chemotherapeutics, targeted therapeutics, and cancer immunotherapeutics) in other BC subtypes, including HER2-positive BC and triple-negative BC.

Acknowledgments
Not applicable.

Author Contributions
Conceptualization: Ho-Sung Lee, In-Hee Lee, Dae-Yeon Lee; Methodology: Ho-Sung Lee, In-Hee Lee, Dae-Yeon Lee; Data collection: Ho-Sung Lee, In-Hee Lee, Kyungae Kang, Sang-In Park, Minho Jung, Seung Gu Yang, Tae-Wook Kwon; Data analysis and investigation: Ho-Sung Lee, In-Hee Lee, Dae-Yeon Lee; Writing: Ho-Sung Lee, In-Hee Lee, Dae-Yeon Lee; All authors read and approved the final manuscript.

Data Statement
All data either generated or analyzed during this study are included in this published article and its supplementary materials file.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval
Not applicable, because this article does not contain any studies with human or animal subjects.

Funding
This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2021R1F1A1049472).

Statement of Informed Consent
Not applicable, because this article does not contain any studies with human or animal subjects.

Statement of Human and Animal Rights
This article does not contain any studies with human or animal subjects.

Trial Registration
Not applicable, because this article does not contain any clinical trials.

Supplemental Material
Supplemental material for this article is available online.

ORCID iD
Dae-Yeon Lee https://orcid.org/0000-0002-3198-9881

References
1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-249.
2. Goldhirsch A, Wood WC, Coates AS, et al. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the primary therapy of early breast cancer 2011. Ann Oncol. 2011;22(8):1736-1747.
3. Patel HK, Bihani T. Selective estrogen receptor modulators (SERMs) and selective estrogen receptor degraders (SERDs) in cancer treatment. Pharmacol Ther. 2018;186:1-24.
4. Awan A, Esfahani K. Endocrine therapy for breast cancer in the primary care setting. Curr Oncol. 2018;25(4):285-291.
5. Ohnishi S, Takeda H. Herbal medicines for the treatment of cancer chemotherapy-induced side effects. Front Pharmacol. 2015;6(14):1-14.
6. Poornima P, Kumar JD, Zhao Q, et al. Network pharmacology of cancer: from understanding of complex interactomes to the design of multi-target specific therapeutics from nature. Pharmacol Res. 2016;111:290-302.
7. Yin SY, Wei WC, Jian FY, et al. Therapeutic applications of herbal medicines for cancer patients. Evid Based Complement Alternat Med. 2013;2013(302426):1-16.
8. Zhu L, Li L, Li Y, et al. Chinese Herbal medicine as an adjunctive therapy for breast cancer: a systematic review and meta-analysis. Evid Based Complement Alternat Med. 2016;2016(9469276):1-18.
9. Lee IH, Lee DY. FDY003 Inhibits colon cancer in a Colo205 xenograft mouse model by decreasing oxidative stress. Pharmacogn Mag. 2019;15(65):675-681.
10. Lee HS, Lee IH, Kang K, et al. Systems pharmacology study of the anticervical cancer mechanisms of FDY003. Nat Prod Commun. 2020;15(12):1-15.
11. Lee HS, Lee IH, Kang K, et al. A network pharmacology study on the molecular mechanisms of FDY003 for breast cancer treatment. Evid Based Complement Alternat Med. 2021;2021(3919143):1-18.
12. Lee WY, Lee CY, Kim YS, et al. The methodological trends of traditional herbal medicine employing network pharmacology. *Molecules*. 2019;9(8):1-15.

13. Lee HS, Lee IH, Park SJ, et al. Network pharmacology-based investigation of the system-level molecular mechanisms of the hematopoietic activity of Samul-Tang, a traditional Korean herbal formula. *Evid Based Complement Alternat Med*. 2020;2020(904809):1-17.

14. He R, Ou S, Chen S, et al. Network pharmacology-based study on the molecular biological mechanism of action for compound Kushen in anti-cancer effect. *Med Sci Monit*. 2020;26(918520):1-15.

15. Mi JL, Liu C, Xu M, et al. Network pharmacology to uncover the molecular mechanisms of action of LeigongTeng for the treatment of nasopharyngeal carcinoma. *Med Sci Monit Basic Res*. 2020;26(923431):1-15.

16. Wang Y, Dong B, Xue W, et al. Anticancer effect of radix astragali on cholangiocarcinoma in vitro and its mechanism via network pharmacology. *Med Sci Monit*. 2020;26(921162):1-20.

17. Xu T, Wang Q, Liu M. A network pharmacology approach to explore the potential mechanisms of Huangqin-Baishao herb pair in treatment of cancer. *Med Sci Monit*. 2020;26(923199):1-11.

18. Zhang SQ, Xu HB, Zhang SJ, et al. Identification of the active compounds and significant pathways of artesimia annua in the treatment of non-small cell lung carcinoma based on network pharmacology. *Med Sci Monit*. 2020;26(923624):1-11.

19. Ru J, Li P, Wang J, et al. TC MSP: a database of systems pharmacology for drug discovery from herbal medicines. *J Cheminform*. 2014;6(13):1-6.

20. Tao W, Li B, Gao S, et al. CancerHSP: anticancer herbs database of systems pharmacology. *Sci Rep*. 2015;5(11481):1-6.

21. Liu Z, Guo F, Wang Y, et al. BATMAN-TCM: a bioinformatics analysis tool for molecular mechanisms of traditional Chinese medicine. *Sci Rep*. 2016;6(21146):1-11.

22. Huang L, Xie D, Yu Y, et al. TC MID 2.0: a comprehensive resource for TCM. *Nucleic Acids Res*. 2018;46(D1):D1117-D1120.

23. Yue SJ, Xin LT, Fan YC, et al. Herb pair Danggui-Honghua: mechanisms underlying blood stasis syndrome by system pharmacology approach. *Sci Rep*. 2017;7(40318):1-15.

24. Kono Y, Iwasaki A, Matsuoka K, et al. Effect of mechanical agitation on cationic liposome transport across an unstirred water layer in CaCo-2 cells. *Bio Pharm Bull*. 2016;39(8):1293-1299.

25. Volpe DA. Variability in CaCo-2 and MDCK cell-based intestinal permeability assays. *J Pharm Sci*. 2008;97(2):712-725.

26. Garcia MN, Flowers C, Cook JD. The CaCo-2 cell culture system can be used as a model to study food iron availability. *J Nutr*. 1996;126(1):251-258.

27. Li Y, Zhang J, Zhang L, et al. Systems pharmacology to decipher the combinational anti-migraine effects of Tianshu formula. *J Ethnopharmacol*. 2015;174:45-56.

28. Zhang J, Li Y, Chen X, et al. Systems pharmacology dissection of multi-scale mechanisms of action for herbal medicines in stroke treatment and prevention. *PLoS One*. 2014;9(8):1-17.

29. Lee AY, Park W, Kang TW, et al. Network pharmacology-based prediction of active compounds and molecular targets in Yijn-Tang acting on hyperlipidemia and atherosclerosis. *J Ethnopharmacol*. 2018;221:151-159.

30. Wang CK, Craik DJ. Cyclic peptide oral bioavailability: lessons from the past. *Biopolymers*. 2016;106(6):901-909.

31. Kim S, Chen J, Cheng T, et al. Pubchem 2019 update: improved access to chemical data. *Nucleic Acids Res*. 2019;47(D1):D1102-D1109.

32. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res*. 2019;47(W1):W357-W364.

33. Wang X, Shen Y, Wang S, et al. Pharmmapper 2017 update: a web server for potential drug target identification with a comprehensive target pharmacophore database. *Nucleic Acids Res*. 2017;45(W1):W356-W360.

34. Szklarczyk D, Santos A, von Mering C, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2015;43(Database issue):D789-D798.

35. Keiser MJ, Roth BL, Armbruster BN, et al. Relating protein pharmacology by ligand chemistry. *Nat Biotechnol*. 2007;25(2):197-206.

36. Yang K, Zeng L, Ge J. Exploring the pharmacological mechanism of Danzhi Xiaoyao powder on ER-positive breast cancer by a network pharmacology approach. *Evid Based Complement Alternat Med*. 2018;2018(5059743):1-21.

37. Pinero J, Bravo A, Queralt-Rosinach N, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic Acids Res*. 2017;45(D1):D383-D389.

38. Zhu F, Han B, Kumar P, et al. Update of TTD: therapeutic target database. *Nucleic Acids Res*. 2010;38(Database issue):D787-D791.

39. Amberger JS, Bocchini CA, Schiettecatte F, et al. OMIM.org: online Mendelian inheritance in man (OMIM(R)), an online catalog of human genes and genetic disorders. *Nucleic Acids Res*. 2015;43(Database issue):D789-D798.

40. Safran M, Dalah I, Alexander J, et al. Genecards version 3: the human gene integrator. *Database*. 2010;2010(2010):1-16.

41. Wishart DS, Feunang YD, Guo AC, et al. Drugbank 5.0: a major update to the Drugbank database for 2018. *Nucleic Acids Res*. 2018;46(D1):D1074-D1082.

42. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2019;47(D1):D607-D613.

43. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498-2504.

44. Babarasi AL, Olvai ZN. Network biology: understanding the cell’s functional organization. *Nat Rev Genet*. 2004;5(2):101-113.

45. Nagy A, Lanczky A, Menyhart O, et al. Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets. *Sci Rep*. 2018;8(1):1-9.
82. Wang M, Wu X, Chai F, et al. Plasma prolactin and breast cancer risk: a meta-analysis. Sci Rep. 2016;6(25998):1-7.
83. Perks CM, Keith AJ, Goodhew KL, et al. Prolactin acts as a potent survival factor for human breast cancer cell lines. Br J Cancer. 2004;91(2):305-311.
84. da Silva PL, do Amaral VC, Gabrielli V, et al. Prolactin promotes breast cancer cell migration through actin cytoskeleton remodeling. Front Endocrinol. 2015;6(186):1-8.
85. Howell SJ, Anderson E, Hunter T, et al. Prolactin receptor antagonism reduces the clonogenic capacity of breast cancer cells and potentiates doxorubicin and paclitaxel cytotoxicity. Breast Cancer Res. 2008;10(4):1-11.
86. Goldberg JE, Schwertfeger KL. Proinflammatory cytokines in breast cancer: mechanisms of action and potential targets for therapeutics. Curr Drug Targets. 2010;11(9):1133-1146.
87. Mercogliano MF, Bruni S, Elizalde PV, et al. Tumor necrosis factor alpha blockade: an opportunity to tackle breast cancer. Front Oncol. 2020;10(584):1-25.
88. Mercurio AM, Lipscomb EA, Bachelder RE. Non-angiogenic functions of VEGF in breast cancer. J Mammary Gland Biol Neoplasia. 2005;10(4):283-290.
89. Perrot-Appleman M, Di Benedetto M. Autocrine functions of VEGF in breast tumor cells: adhesion, survival, migration and invasion. Cell Adh Migr. 2012;6(6):547-553.
90. Lipponen P. Apoptosis in breast cancer: relationship with other pathological parameters. Endocr Relat Cancer. 1999;6(1):13-16.
91. Pare R, Yang T, Shin JS, et al. The significance of the senescence pathway in breast cancer progression. J Clin Pathol. 2013;66(6):491-495.
92. Parton M, Dowsett M, Smith I. Studies of apoptosis in breast cancer. Br Med J. 2001;322(7301):1528-1532.
93. Sutherland RL, Musgrove EA. Cyclins and breast cancer. J Mammary Gland Biol Neoplasia. 2004;9(1):95-104.
94. Musgrove EA, Sutherland RL. Biological determinants of endocrine resistance in breast cancer. Nat Rev Cancer. 2009;9(9):631-643.
95. Clark AS, West K, Streicher S, et al. Constitutive and inducible Akt activity promotes resistance to chemotherapy, trastuzumab, or tamoxifen in breast cancer cells. Mol Cancer Ther. 2002;1(9):707-717.
96. Clarke R, Tyson JJ, Dixon JM. Endocrine resistance in breast cancer-An overview and update. Mol Cell Endocrinol. 2015;418(Pt 3):220-234.
97. Eckstein N. Platinum resistance in breast and ovarian cancer cell lines. J Exp Clin Cancer Res. 2011;30(91):1-11.
98. Decatur MP, Sundar S, O’Byrne KJ. Platinum-based chemotherapy in metastatic breast cancer: current status. Cancer Treat Rev. 2004;30(1):53-81.
99. Pohlmann PR, Mayer IA, Mernagh R. Resistance to Trastuzumab in breast cancer. Clin Cancer Res. 2009;15(24):7479-7491.
100. Osborne CK, Schiff R. Mechanisms of endocrine resistance in breast cancer. Annu Rev Med. 2011;62:233-247.
101. Caballero J. The latest automated docking technologies for novel drug discovery. Expert Opin Drug Discov. 2021;16(6):625-645.
102. Pinzi I, Rastelli G. Molecular docking: shifting paradigms in drug discovery. Int J Mol Sci. 2019;20(18):1-23.
103. Kaur T, Madgulkar A, Bhalekar M, et al. Molecular docking in formulation and development. Curr Drug Discovery Technol. 2019;16(1):30-39.
104. Wang Z, Sun H, Yao X, et al. Comprehensive evaluation of ten docking programs on a diverse set of protein-ligand complexes: the prediction accuracy of sampling power and scoring power. Phys Chem Chem Phys. 2016;18(18):12964-12975.
105. Wang S, Lin H, Cong W. Chinese Medicines improve perimenopausal symptoms induced by surgery, chemoradiotherapy, or endocrine treatment for breast cancer. Front Pharmacol. 2019;10(174):1-16.
106. You I, An R, Liang K, et al. Anti-breast cancer agents from Chinese herbal medicines. Mini Rev Med Chem. 2013;13(1):101-105.
107. Vestey SB, Sen C, Calder CJ, et al. Activated Akt expression in breast cancer: correlation with p53, Hdm2 and patient outcome. Eur J Cancer. 2005;41(7):1017-1025.
108. Perez-Tenorio G, Stal O. Southeast Sweden breast cancer G. Activation of AKT/PKB in breast cancer predicts a worse outcome among endocrine treated patients. Br J Cancer. 2002;86(4):540-545.
109. Kirkegaard T, Witton CJ, McGlynn LM, et al. Akt Activation predicts outcome in breast cancer patients treated with tamoxifen. J Pathol. 2005;207(2):139-146.
110. Tokunaga E, Kataoka A, Kinura Y, et al. The association between Akt activation and resistance to hormone therapy in metastatic breast cancer. Eur J Cancer. 2006;42(5):629-635.
111. Davis NM, Sokolosky M, Stadelman K, et al. Deregulation of the EGFR/PI3K/PTEN/Akt/mTORC1 pathway in breast cancer: possibilities for therapeutic intervention. OncoTarget. 2014;5(13):4603-4650.
112. Mueller KL, Powell K, Madden JM, et al. EGFR Tyrosine 845 phosphorylation-depended proliferation and transformation of breast cancer cells require activation of p38 MAPK. Transl Oncol. 2012;5(5):327-334.
113. Dilhe I, Bendahl PO, Grabau D, et al. Epidermal growth factor receptor (EGFR) and the estrogen receptor modulator amplified in breast cancer (AIB1) for predicting clinical outcome after adjuvant tamoxifen in breast cancer. Breast Cancer Res Treat. 2008;109(2):255-262.
114. Ghayad SE, Cohen PA. Inhibitors of the PI3K/Akt/mTOR pathway: new hope for breast cancer patients. Recent Pat Anticancer Drug Discov. 2010;5(1):29-57.
115. Verbeek BS, Adriaansen-Slot SS, Vroom TM, et al. Overexpression of EGFR and c-erbB2 causes enhanced cell migration in human breast cancer cells and NIH3T3 fibroblasts. FEBS Lett. 1998;425(1):145-150.
116. Kallergi G, Angelaki S, Kalykaki A, et al. Phosphorylated EGFR and PI3K/Akt signaling kinases are expressed in circulating tumor cells of breast cancer patients. Breast Cancer Res. 2008;10(5):1-11.
117. Paplomata E, O'Regan R. The PI3K/AKT/mTOR pathway in breast cancer: targets, trials and biomarkers. *Thor Adsc Med Oncol*. 2014;6(4):154-166.

118. Al-Bahiani SM, Lakhakia R, Al-Jaaid SS, et al. Correlation of expression of Akt1 and E2F1 and their phosphorylated forms in breast cancer patients with clinicopathological parameters. *J Mol Histol*. 2021;52(3):621-633.

119. Deng L, Zhu X, Sun Y, et al. Prevalence and prognostic role of PIK3CA/akt1 mutations in Chinese breast cancer patients. *Cancer Res Treat*. 2019;51(1):128-140.

120. Wang Y, Wu Z, Zhou L, et al. The impact of EGFR gene polymorphisms on the response and toxicity derived from neoadjuvant chemotherapy for breast cancer. *Gland Surg*. 2020;9(4):925-935.

121. Albert JM, Kim KW, Cao C, et al. Targeting the Akt/mammalian target of rapamycin pathway for radiosensitization of breast cancer. *J Natl Cancer Inst*. 2006;98(5):1183-1189.

122. Zhang W, Liu X, Liu S, et al. Androgen receptor/let-7a signaling regulates breast tumor-initiating cells. *Oncotarget*. 2018;9(3):3690-3703.

123. Kensler KH, Poole EM, Heng YJ, et al. Androgen receptor expression and breast cancer survival: results from the Nurses' Health Studies. *J Natl Cancer Inst*. 2019;111(7):700-708.

124. Okano M, Oishi M, Butash AL, et al. Estrogen receptor positive breast cancer with high expression of androgen receptor has less cytolytic activity and worse response to neoadjuvant chemotherapy but better survival. *Int J Mol Sci*. 2019;20(11):1-11.

125. Hayashi SI, Eguchi H, Tanimoto K, et al. The expression and function of estrogen receptor alpha and beta in human breast cancer and its clinical application. *Endocr Relat Cancer*. 2003;10(2):193-202.

126. Kim C, Tang G, Pogue-Geile KL, et al. Acacetin-induced apoptosis of human breast cancer MCF-7 cells involves caspase cascade, mitochondrial-mediated death signaling and SAPK/JNK1/2-e-Jun activation. *Mult Cells*. 2007;24(1):95-104.

127. Ning L, Ma H, Jiang Z, et al. Curcumol suppresses breast cancer cell metastasis by inhibiting MMP-9 Via JNK1/2 and Akt-dependent NF-kappaB signaling pathways. *Integr Cancer Ther*. 2016;15(2):216-225.

128. Sun M, Isaacs GD, Hahn N, et al. Estrogen regulates JNK1 genomic localization to control gene expression and cell growth in breast cancer cells. *Mutat Res Rev Sci Rep*. 2016;5(2246):1-6.

129. Reintert T, Goncalves R, Bines J. Implications of ESR1 mutations in hormone receptor-positive breast cancer. *Curr Treat Options Oncol*. 2018;19(5):1-13.

130. Robinson DR, Wu YM, Vais P, et al. Activating ESR1 mutations in hormone-resistant metastatic breast cancer. *Nat Genet*. 2013;45(12):1446-1451.

131. Tripicianis G, Papadopoulos E, Anagnostopoulos K, et al. Coexpression of IL-6 and TNF-alpha: prognostic significance on breast cancer outcome. *Nonplasma*. 2014;61(2):205-212.

132. Abana CO, Bingham BS, Cho JH, et al. IL-6 variant is associated with metastasis in breast cancer patients. *PLoS One*. 2017;12(7):1-15.

133. Casneuf T, Axel AE, King P, et al. Interleukin-6 is a potential therapeutic target in interleukin-6 dependent, estrogen receptor-alpha-positive breast cancer. *Breast Cancer (Dove Med Press)*. 2016;8:13-27.

134. Dethlefsen C, Hojfeldt G, Hojman P. The role of intratumoral and systemic IL-6 in breast cancer. *Breast Cancer Res Treat*. 2013;138(3):657-664.

135. Knupfer H, Reiss R. Significance of interleukin-6 (IL-6) in breast cancer (review). *Breast Cancer Res Treat*. 2007;102(2):129-135.

136. DeMichele A, Gray R, Horn M, et al. Host genetic variants in the interleukin-6 promoter predict poor outcome in patients with estrogen receptor-positive, node-positive breast cancer. *Cancer Res*. 2009;69(10):4184-4191.

137. DeMichele A, Martin AM, Mick R, et al. Interleukin-6 -174G-->C polymorphism is associated with improved outcome in high-risk breast cancer. *Cancer Res*. 2003;63(22):8051-8056.

138. Lin S, Gan Z, Han K, et al. Interleukin-6 as a prognostic marker for breast cancer: a meta-analysis. *Tumori*. 2015;101(5):535-541.

139. Starkweather A. Increased interleukin-6 activity associated with painful chemotherapy-induced peripheral neuropathy in women after breast cancer treatment. *Nurs Res Pract*. 2010;2010(281531):281531.

140. Shim HY, Park JH, Paik HD, et al. Acacetin-induced apoptosis of human breast cancer MCF-7 cells involves caspase cascade, mitochondrial-mediated death signaling and SAPK/JNK1/2-e-Jun activation. *Mult Cells*. 2007;24(1):95-104.

141. Ding L, Ma H, Jiang Z, et al. Curcumol suppresses breast cancer cell metastasis by inhibiting MMP-9 via JNK1/2 and Akt-dependent NF-kappaB signaling pathways. *Integr Cancer Ther*. 2016;15(2):216-225.

142. Sun M, Isaacs GD, Hahn N, et al. Estrogen regulates JNK1 genomic localization to control gene expression and cell growth in breast cancer cells. *Mutat Res Rev Sci Rep*. 2016;5(2246):1-6.

143. Lisanti MP, Tsirigos A, Pavilek S, et al. JNK1 Stress signaling is hyper-activated in high breast density and the tumor stroma: connecting fibrosis, inflammation, and stemness for cancer prevention. *Cell Cycle*. 2014;13(4):580-599.

144. Zhang M, Wang Y, Jiang L, et al. LncRNA CBR3-AS1 regulates breast cancer drug sensitivity as a competing endogenous RNA through the JNK1/MEK4-mediated MAPK signal pathway. *J Exp Clin Cancer Res*. 2021;40(1):1-14.

145. Su L, Jiang Y, Xu Y, et al. Xihuang pill promotes apoptosis of Treg cells in the tumor microenvironment in 4T1 mouse breast cancer by upregulating MEKK1/SEK1/JNK1/AP-1 pathway. *Biomed Pharmacother*. 2018;102:1111-1119.

146. Miller LD, Smeds J, George J, et al. An expression signature for breast cancer patients with clinicopathological parameters. *PLoS One*. 2013;8(2):e53550.

147. Yang P, Du CW, Kwan M, et al. The impact of p53 in predicting clinical outcome of breast cancer patients with visceral metastasis. *Biomed Pharmacother*. 2018;102:1-6.

148. Wellenstein MD, Coffelt SB, Duits DEM, et al. Loss of p53 triggers WNT-dependent systemic inflammation to drive breast cancer metastasis. *Nature*. 2019;572(7770):538-542.
149. Lim LY, Vidnovic N, Ellisen LW, et al. Mutant p53 mediates survival of breast cancer cells. Br J Cancer. 2009;101(9):1606-1612.

150. Yamashita H, Toyama T, Nishio M, et al. P53 protein accumulation predicts resistance to endocrine therapy and decreased post-relapse survival in metastatic breast cancer. Breast Cancer Res. 2006;8(4):1-8.

151. Walerych D, Napoli M, Collavin L, et al. The rebel angel: mutant p53 as the driving oncogene in breast cancer. Carcinogenesis. 2012;33(11):2007-2017.

152. Na B, Yu W, Withers T, et al. Therapeutic targeting of BRCA1 and TP53 mutant breast cancer through mutant p53 reactivation. NPJ Breast Cancer. 2019;5(14):1-10.

153. Baker L, Quinlan PR, Patten N, et al. P53 mutation, deprivation and poor prognosis in primary breast cancer. Br J Cancer. 2010;102(4):719-726.

154. Kandioler-Eckersberger D, Ludwig C, Rudas M, et al. TP53 as the driving oncogene in breast cancer. Carcinogenesis. 2010;31(4):154-160.

155. Baker L, Quinlan PR, Patten N, et al. P53 mutation, deprivation and poor prognosis in primary breast cancer. Br J Cancer. 2010;102(4):719-726.

156. Liang Y, Brekken RA, Hyder SM. Vascular endothelial growth factor induces proliferation of breast cancer cells and inhibits the anti-proliferative activity of anti-hormones. Endocr Relat Cancer. 2006;13(3):905-919.

157. Luo M, Hou L, Li J, et al. VEGF/NRP-1-axis promotes progression of breast cancer via enhancement of epithelial-mesenchymal transition and activation of NF-kappaB and beta-catenin. Cancer Lett. 2016;373(1):1-11.

158. Balasubramanian SP, Cox A, Cross SS, et al. Influence of VEGF-A gene variation and protein levels in breast cancer susceptibility and severity. Int J Cancer. 2007;121(5):1009-1016.

159. Etienne-Grimaldi MC, Formento P, Degeorges A, et al. Prospective analysis of the impact of VEGF-A gene polymorphisms on the pharmacodynamics of bevacizumab-based therapy in metastatic breast cancer patients. Br J Clin Pharmacol. 2011;71(6):921-928.

160. Lu H, Shu XO, Cui Y, et al. Association of genetic polymorphisms in the VEGF gene with breast cancer survival. Cancer Res. 2005;65(12):5015-5019.

161. Sanchez BC, Sundqvist M, Fohlin H, et al. Prolonged tamoxifen treatment increases relapse-free survival for patients with primary breast cancer expressing high levels of VEGF. Eur J Cancer. 2010;46(9):1580-1587.

162. Santos LV, Cruz MR, Lopes Gde L, et al. VEGF-A levels in bevacizumab-treated breast cancer patients: a systematic review and meta-analysis. Breast Cancer Res Treat. 2015;151(3):481-489.

163. Kim JH, Kim DH, You JH, et al. Comparison of cytotoxicity and immune activities between natural and tissue cultured plant in Artemisia Capillaris Thunb. Korean J Med Crop Sci. 2005;13(4):154-160.

164. Chen C, Wang ML, Jin C, et al. Cordyceps militaris polysaccharide triggers apoptosis and G0/G1 cell arrest in cancer cells. J Asia Pac Entomol. 2015;18(3):433-438.

165. Song J, Wang Y, Teng M, et al. Cordyceps militaris induces tumor cell death via the caspasedependent mitochondrial pathway in HepG2 and MCF7 cells. Med Med Rep. 2016;13(6):5132-5140.

166. Lee SO, Jeong YJ, Kim M, et al. Suppression of PMA-induced tumor cell invasion by capallarisis via the inhibition of NF-kappaB-dependent MMP-9 expression. Biochem Biophys Res Commun. 2008;366(4):1019-1024.

167. Noh EM, Youn HJ, Jung SH, et al. Cordycepin inhibits TPA-induced matrix metalloproteinase-9 expression by suppressing the MAPK/AP-1 pathway in MCF-7 human breast cancer cells. Int J Mol Med. 2010;25(2):255-260.

168. Wang D, Zhang Y, Lu J, et al. Cordycepin, a natural antineoplastic agent, induces apoptosis of breast cancer cells via caspase-dependent pathways. Nat Prod Commun. 2016;11(1):63-68.

169. Choi S, Lim MH, Kim KM, et al. Cordycepin-induced apoptosis and autophagy in breast cancer cells are independent of the estrogen receptor. Tissue Cell Pharmacol. 2011;257(2):165-173.

170. Dong J, Li Y, Xiao H, et al. Cordycepin sensitizes breast cancer cells toward irradiation through elevating ROS production involving Nrf2. Tissue Cell Pharmacol. 2019;364:12-21.

171. Zhang HW, Hu JJ, Fu RQ, et al. Flavonoids inhibit cell proliferation and induce apoptosis and autophagy through downregulation of PI3K-gamma mediated PI3 K/AKT/mTOR/p70S6K/ULK signaling pathway in human breast cancer cells. Sci Rep. 2018;8(1):1-13.

172. Li Y, Hong J, Li H, et al. Genkwanin nanosuspensions: a novel and potential antitumor drug in breast carcinoma therapy. Drug Deliv. 2017;24(1):1491-1500.

173. Wu Q, Koo PA, Shao H, et al. Differential effects of quercetin and two of its derivatives, isoquercetin and isorhamnetin-3-glucuronide, in inhibiting the proliferation of human breast-cancer MCF-7 cells. J Agri Food Chem. 2018;66(27):7181-7189.

174. Alvarez-Sala A, Attanzio A, Tesoriere L, et al. Apoptotic effect of a phytosterol-ingredient and its main phytosterol (beta-sitosterol) in human cancer cell lines. Int J Food Sci Nutr. 2019;70(3):323-334.

175. Awad AB, Barta SL, Fink CS, Bradford PG. Beta-sitosterol enhances tamoxifen effectiveness on breast cancer cells by affecting ceramide metabolism. Mol Nutr Food Res. 2008;52(4):419-426.

176. Azevedo C, Correia-Branco A, Araujo JR, et al. The chemopreventive effect of the dietary compound kaempferol on the MCF-7 human breast cancer cell line is dependent on inhibition of glucose cellular uptake. Nutr Cancer. 2015;67(3):504-513.

177. Chou CC, Yang JS, Lu HF, et al. Quercetin-mediated cell cycle arrest and apoptosis involving activation of a caspase cascade through the mitochondrial pathway in human breast cancer MCF-7 cells. Arch Pharm Res. 2010;33(8):1181-1191.

178. Cook MT, Liang Y, Besch-Williford C, et al. Luteolin inhibits progesterin-dependent angiogenesis, stem cell-like characteristics, and growth of human breast cancer xenografts. SpringerPlus. 2015;4(444):1-16.
179. Duo J, Ying GG, Wang GW, et al. Quercetin inhibits human breast cancer cell proliferation and induces apoptosis via Bcl-2 and Bax regulation. *Mol Med Rep*. 2012;5(6):1453-1456.

180. Hung H. Inhibition of estrogen receptor alpha expression and function in MCF-7 cells by kaempferol. *J Cell Physiol*. 2004;198(2):197-208.

181. Jia L, Huang S, Yin X, et al. Quercetin suppresses the mobility of breast cancer by suppressing glycolysis through Akt-mTOR pathway mediated autophagy induction. *Life Sci*. 2018;208:123-30.

182. Lee GA, Choi KC, Hwang KA. Kaempferol, a phytoestrogen, suppressed triclosan-induced epithelial-mesenchymal transition and metastatic-related behaviors of MCF-7 breast cancer cells. *Environ Toxicol Pharmacol*. 2017;49:48-57.

183. Li X, Zhou N, Wang J, et al. Quercetin suppresses breast cancer stem cells (CD44(+) /CD24(−)) by inhibiting the PI3K/Akt/mTOR-signaling pathway. *Life Sci*. 2018;196:56-62.

184. Lin CH, Chang CY, Lee KR, et al. Flavones inhibit breast cancer proliferation through the Akt/FOXO3a signaling pathway. *BMC Cancer*. 2015;15(958):1-12.

185. Lin CW, Hou WC, Shen SC, et al. Quercetin inhibition of tumor invasion via suppressing PKC delta/ERK/AP-1-dependent matrix metalloproteinase-9 activation in breast carcinoma cells. *Carcinogenesis*. 2008;29(9):1807-1815.

186. Park SH, Ham S, Kwon TH, et al. Luteolin induces cell cycle arrest and apoptosis through extrinsic and intrinsic signaling pathways in MCF-7 breast cancer cells. *J Environ Pathol Toxicol Oncol*. 2014;33(3):219-231.

187. Sui JQ, Xie KP, Xie MJ. Inhibitory effect of luteolin on the proliferation of human breast cancer cell lines induced by epidermal growth factor. *Sheng Li Xue Bao*. 2016;68(1):27-34.

188. Tao SF, He HF, Chen Q. Quercetin inhibits proliferation and invasion acts by up-regulating miR-146a in human breast cancer cells. *Med Cell Biochem*. 2015;402(1-2):93-100.

189. Wang LM, Xie KP, Huo HN, et al. Luteolin inhibits proliferation induced by IGF-1 pathway dependent ERalpha in human breast cancer MCF-7 cells. *Asian Pac J Cancer Prev*. 2012;13(4):1431-1437.

190. Zhao X, Wang Q, Yang S, et al. Quercetin inhibits angiogenesis by targeting calcineurin in the xenograft model of human breast cancer. *Eur J Pharmacol*. 2016;781:60-68.

191. Li S, Yuan S, Zhao Q, et al. Quercetin enhances chemotherapeutic effect of doxorubicin against human breast cancer cells while reducing toxic side effects of it. *Biomed Pharmacother*. 2018;100:441-447.

192. Tu SH, Ho CT, Liu MF, et al. Luteolin sensitises drug-resistant human breast cancer cells to tamoxifen via the inhibition of cyclin E2 expression. *Food Chem*. 2013;141(2):1553-1561.

193. Soltanian S, Riahirad H, Pabarja A, et al. Kaempferol and docetaxel diminish side population and down-regulate some cancer stem cell markers in breast cancer cell line MCF-7. *Biochim Biophys Acta*. 2017;41(2-3):33-40.

194. Sun DW, Zhang HD, Mao L, et al. Luteolin inhibits breast cancer development and progression in vitro and in vivo by suppressing notch signaling and regulating MiRNAs. *Cell Physiol Biochem*. 2015;37(5):1693-1711.

195. Agrawal PK, Agrawal C, Blunden GJNPC. Quercetin: antiviral significance and possible COVID-19 integrative considerations. *Nat Prod Commun*. 2020;15(12):1-10.

196. Oh SJ, Kim O, Lee JS, et al. Inhibition of angiogenesis by quercetin in tamoxifen-resistant breast cancer cells. *Food Chem Toxicol*. 2010;48(11):3227-3234.

197. Ravishankar D, Watson KA, Boateng SY, et al. Exploring quercetin and luteolin derivatives as antiangiogenic agents. *Eur J Med Chem*. 2015;97:259-274.

198. Xiao X, Shi D, Liu I, et al. Quercetin suppresses cyclooxygenase-2 expression and angiogenesis through inactivation of P300 signaling. *PLoS One*. 2011;6(8):1-10.

199. Li SZ, Li K, Zhang JH, et al. The effect of quercetin on doxorubicin cytotoxicity in human breast cancer cells. *Anticancer Agents Med Chem*. 2013;13(2):352-355.