Soy Foods Might Weaken the Sensitivity of Tamoxifen in Premenopausal Patients With Lumina A Subtype of Breast Cancer

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Abstract

Epidemiologic studies held that soy foods are beneficial to breast diseases, but the impact of soy foods on breast diseases is still controversial. In premenopausal patients with Lumina A subtypes of breast cancer, 14 samples were analyzed by GEO2R. Soy foods altered the estrogen receptor-related gene profile in Lumina A subtypes of breast cancer dramatically. Soy foods might weaken the sensitivity of tamoxifen and improve the curative effect of neoadjuvant chemotherapy.

Background: Based on estrogen active substances, many women consume soy foods in the belief that it could prevent breast cancer (BC). Women with different molecular subtypes would be likely to have diverse reactions to soy foods, especially those with the estrogen-receptor-positive (ER^+) subtype. The aim of the current study is to identify the differentially expressed genes (DEGs) on soy foods in premenopausal patients with Lumina A subtype of BC (LABC) after soy food treatment, and to further investigate the critical molecule change. **Materials and Methods:** GSE58792 retrieved from Gene Expression Omnibus was analyzed to obtain DEGs using GEO2R. Gene Ontology and pathway enrichment analysis were performed using FunRich and GeneMINIA. Overall survival of critical genes was performed by the Kaplan-Meier plotter online tool. **Results:** A total of 108 DEGs were obtained from the dataset, among which 35 were up-regulated and 73 down-regulated. Soy foods significantly reduced the expression of TFF3, TFF1, GATA3, and ESR1, which were related to the activity of the ER-related pathway and the sensitivity of tamoxifen. Furthermore, the lower expressions of TOX3, FSIP1, ESR1, and CLGN were related to prolonged survival time of patients with BC. The most significant signaling pathways were epithelial-to-mesenchymal transition in up-regulated DEGs, mesenchymal-to-epithelial transition, and mammary gland alveolus development in down-regulated DEGs, which were all related to the development and prognosis of BC. **Conclusions:** Soy foods could dramatically alter the ER-related gene profile in LABC. Particularly, down-regulated DEGs of TFF3, TFF1, GATA3, and ESR1 might weaken the sensitivity of tamoxifen and increase the efficacy of neoadjuvant chemotherapy in premenopausal patients with LABC.

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Introduction

Breast cancer is the most common type of gynecologic malignancy in women worldwide.[1] Although the mortality rate is decreasing owing to the progresses in screening, diagnosis, and treatment, the incidence of breast cancer is still increasing, and tumor recurrence and metastatic relapse remain the major problems contributing to the death rate.[2] Epidemiologic studies indicate that high dietary intake of soy foods might be significantly associated with a decreased risk of death and recurrence in patients with breast cancer.[3,4] For this reason, most women consume soy in the belief that it prevents breast cancer and treats the gynecologic disease. However, breast cancer is a heterogeneous group of diseases that consists of several molecular subtypes with different biological behaviors and different molecular-targeted therapeutic medicines.[5-7] Consuming soy foods might cause a variety of reactions in women with different breast cancer subtypes.

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Soy foods contain numerous biologically active compounds, such as genistein and isoflavones, which have estrogenic activity.[8] On this basis, having soy foods when an estrogen receptor-positive (ER^+) breast cancer is present may result in a special effect. Andrade et al demonstrated that genistein at physiological concentrations, either in purified glycoside or aglycone forms or from soy-based sources, stimulates the growth of ER^+ breast cancer and the expression of several ER target genes.[9] And long-term consumption of low genistein doses ($\leq$ 500 ppm, dietary level) promotes MCF-7 tumor growth and results in genistein-induced nonregressing tumors with more aggressive and advanced growth phenotypes.[10] Moreover, genistein is proved to block the inhibitory effects of tamoxifen.[11-14] Nevertheless, Chen et al suggested that genistein inhibited cell proliferation by inactivating the IGF-1R-PI3 K/Akt pathway and decreasing the Bcl-2/Bax mRNA and protein expressions.[15] Moreover, Liu et al showed that exposure to soy foods in MCF-7(ER^+) could prevent isoflavones from stimulating MCF-7 tumor growth in athymic nude mice, indicating that other bioactive compounds in soy can negate the estrogenic properties of isoflavones.[16] There are conflicting results on the impact of soy after breast cancer. Thus, the complex relationship between soy intake and breast cancer remains to be established. Shike et al[17] have demonstrated that soy intake could alter breast cancer-related gene expression including FGFR2, which might drive cancer growth**.** Thus, we conducted a bioinformatic analysis to investigate differentially expressed genes (DEGs) on soy foods in premenopausal patients with Luminal A (ER^+) breast cancer (LABC), the most common type of breast cancer that accounts for 40% to 45% of all types of breast cancer, and was low-grade, ER^+, and observably benefited from endocrine therapy.[18,19] The present study aims to identify the DEGs in premenopausal patients with LABC with soy or non-soy food treatments, and to investigate the critical molecule change after soy food intervention for developing novel approaches for prevention and therapy.

Materials and Methods

Data Acquisition and Identification of DEGs

The transcription profile of GSE58792 that contained 14 premenopausal LABC samples was obtained from the Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) database (Table 1). This dataset, based on the GPL570 Affymetrix Human Genome U133 Plus 2.0 Array, included 14 pre-menopausal LABCs samples. GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/) was applied to screen DEGs between soy foods and placebo treatment samples. DEGs with *P*-values less than .05 and log fold change (logFC) > 1.5 or < -1.5 were selected. GEO2R is an interactive tool that allows users to compare 2 groups of samples in a GEO series to identify DEGs under the same experimental conditions. In total, over 90 percent of GEO data can be accessed and analyzed in this way, and results are presented as a table of genes in sequence of significance and may be viewed as profile graphs.[20]

Significant Functions and Pathway Enrichment Analysis

Using FunRich software, DEGs between the 2 sample types were enriched to identify critical pathways involved in tumor development and metastasis. Pathways with *P*-value $< .05$ were considered to be statistically significant.

Predicted Protein Interaction (PPI) Network Construction

High-quality protein interaction networks can provide key insights into the functional and biological properties of cellular systems. The GeneMANIA (http://genemania.org/) database is an online tool of known and PPIs, including physical and indirect functional associations.[21]

Results

Identification of DEGs

Based on GSE58792 dataset and the GEO2R, a total of 108 DEGs were identified between the soy food intake group and the placebo group in the samples from the premenopausal patients, among which 35 were up-regulated and 73 down-regulated. Some up-regulated genes (eg, FOXC1, MMP1, and MMP7) are associated with invasion and metastasis of breast cancer, whereas parts of down-regulated genes (eg, TOX3, FSIP1, ESR1, and CLGN) were related to the increase of survival time. Interestingly, most significantly down-regulated genes are ER-related genes. The top 65 DEGs are displayed in Table 2 and Table 3.

GO and Pathway Enrichment Analysis

To reveal the biological significance of DEGs, GO functional and pathway enrichment analyses were performed using FunRich software. As demonstrated in Table 4, based on the biological pathway enrichment analysis, the up-regulated DEGs (COL6A2, CRYAB, TUBB6) were identified to be significantly enriched in epithelial-to-mesenchymal transition (EMT), and S100A8 in endogenous TLR signaling, whereas down-regulated DEGs (NRIP1, FOXA1, TFF1, ESR1, ERBB4, AR, TOX3, TFF3) were involved in FOXA transcription factor networks, nuclear estrogen receptor alpha network, erbB receptor signaling network, androgen receptor (AR), and mesenchymal-to-epithelial transition (MET). Furthermore, these signaling pathways were closely related to the survival time of patients with breast cancer. In terms of biological process, up-regulated DEGs (KRT16, MAP2, FSCN1, EMP1, COL6A2, COL3A1, TUBB6) were mainly enriched in the cell growth and/or maintenance; whereas down-regulated DEGs (AR, IGF1R, ERBB4, TFF1, BMPR1B) were involved in cell communication and signal transduction.

PPI Network Analysis of DEGs

The PPI network with significant gene pairs was visualized using GeneMANIA. The combined score over 0.9 was selected as the

Table 1 Fourteen Premenopausal LABC Samples

Placebo Treatment	Soy Food Treatment
GSM1419625	GSM1419651
GSM1419631	GSM1419655
GSM1419636	GSM1419656
GSM1419654	GSM1419662
GSM1419661	GSM1419666
GSM1419664	GSM1419667
GSM1419673	GSM1419670

Abbreviation: LABC = laminal A breast cancer.

Table 2 Up-regulated DEGs in Premenopausal Patients With LABC

Gene Symbol	*P* Value	LogFC	Gene Symbol	*P* Value	LogFC
FSCN1	.000357	1.78	MMP7	.012651	1.77
BACE2	.001507	1.75	EN1	.016247	2.17
PXDN	.001875	1.65	KRT16	.01838	2.13
NFE2L3	.002028	1.74	S100A8	.019187	2.39
MAP2	.002151	1.95	ZIC1	.019927	1.52
COL6A2	.003201	1.65	APOBEC3B	.021469	1.91
TMEM64	.003399	1.69	GBP1	.022112	1.59
LY6K	.003683	2.29	RARRES1	.02249	2.21
TUBB6	.004041	1.5	KCNN4	.026104	1.51
VGLL3	.005038	1.69	CRYAB	.029411	1.59
PAQR5	.007406	1.57	INHBA	.035	1.5
B3GNT5	.008159	1.7	HRASLS	.036249	1.63
PGBD5	.008336	2.34	MUC16	.038796	1.61
EMP1	.008368	1.68	LCN2	.040059	1.98
FOXC1	.010332	2.85	CALML5	.042234	2.52
MPZL2	.010611	1.85	SLC4A11	.043265	1.86
COL3A1	.010777	1.53	MMP1	.0435	2.08
EP300-AS1	.012141	1.69			

Abbreviations: DEGs = differentially expressed genes; LABC = laminal A breast cancer.

threshold. Then, a total of 9 genes (FOXC1, MMP1, MMP7, FSCN1, GATA3, TFF3, TFF1, TOX3 and ESR1) were selected as hub genes to illuminate the biological significance of gene modules in BC (Figure 1).

Survival Analysis of Critical Genes

The prognostic value of 24 critical genes was analyzed in Kaplan-Meier plotter. Overall survival for patients with breast cancer (ER^+, human epidermal growth factor receptor 2-negative) was obtained according to the up- and down-regulated DEGs. It showed that

Table 3 Parts of Down-regulated DEGs in Premenopausal Patients With LABC

Gene Symbol	*P* Value	LogFC	Gene Symbol	*P* Value	LogFC
C1orf168	.000028	−1.98	FOXA1	.009229	−2.13
UGDH	.000048	−1.88	BMPR1B	.010648	−3.44
FAR2P3	.000511	−2.52	PLAT	.011077	−1.88
DYNLRB2	.000738	−1.92	AR	.014006	−2.39
TOX3	.000992	−3.21	FSIP1	.014119	−2.54
ERBB4	.0012	−2.6	ESR1	.015333	−3.54
TFF3	.001331	−2.99	GATA3	.017819	−3.06
ACADSB	.001614	−2.28	PKIB	.018425	−2.48
DNAJC12	.001864	−3.1	CPB1	.019923	−3.31
TMEM144	.002263	−1.51	MYB	.02176	−1.83
SLC7A2	.003515	−3.34	BCL2	.025969	−1.68
PSD3	.004526	−2.39	NRIP1	.029124	−1.57
LINC01087	.0049	−2.36	TFF1	.030095	−2.52
IGF1R	.007264	−1.68	COL4A5	.034288	−1.83
SLC39A6	.008051	−2.57	GREB1	.039618	−2.56

Abbreviations: DEGs = differentially expressed genes; LABC = laminal A breast cancer.

Table 4 Gene Ontology and Pathway Enrichment Analysis for DEGs

Category	*P* Value	Count
High DEGs: pathway-epithelial-to-mesenchymal transition	.005759	3
High DEGs: process-cell growth and/or maintenance	.004272	7
Low DEGs: pathway-FOXA transcription factor networks	2.62E-06	6
Low DEGs: pathway-validated nuclear estrogen receptor alpha network	.000283	4
Low DEGs: pathway-ErbB receptor signaling network	.008346	13
Low DEGs: Pathway-androgen receptor	.007026	3
Low DEGs: pathway-mesenchymal-to-epithelial transition	.000101	7
Low DEGs: process-cell communication	.019848	20
Low DEGs: process-signal transduction	.008928	22

Abbreviation: DEGs = differentially expressed genes.

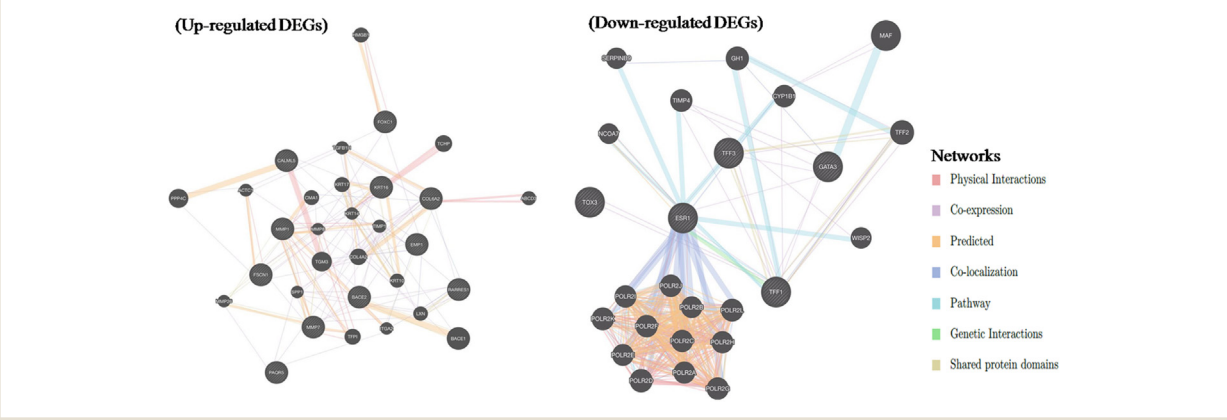
down-regulated DEG of *TOX3* (hazard ratio [HR], 2.11; 95% confidence interval [CI], 1.43-3.11; log rank $P = .00012$) was related to better overall survival, as well as *FSIP1* (HR, 1.61; 95% CI, 1.08-2.41; log rank $P = .018$), *ESR1* (HR, 1.53; 95% CI, 1.05-2.21; log rank $P = .024$), and *CLGN* (HR, 1.49; 95% CI, 1.03-2.17; log rank $P = .034$), whereas up-regulated DEGs such as MMP1 (HR, 1.61; 95% CI, 1.37-1.9; $P = 1.1E-8$), and MMP7 (HR, 1.18; 85% CI, 1-1.39; log rank $P = .049$) were related to worse overall survival (Figure 2).

Discussion

Breast cancer has been classified into several molecular subtypes based on gene expression profiles.[22] The ER^+ subtypes, known as luminal A and luminal B, are the most predominant molecular subtypes of breast cancer, and present with good prognosis and better response to endocrine therapies than ER-negative (ER^-) breast cancer.[23] Different molecular subtypes of breast cancer have diverse reactions to the same substance. Many studies held that patients with breast cancer eat more soy food to reduce the mortality rate of breast cancer.[24,25] However, the breast cancer subtypes and/or hormonal status were always ignored in those works. To our knowledge, this is the first report to analyze gene expression profiles between soy and placebo in patients with LABC. We retrieved a dataset of controlled trials on soy foods to analyze the DEGs and found that soy foods could cause alterations in gene expression in LABC patients.

The impact of soy foods on breast carcinogenesis is very likely to be remarkable. One hundred eight DEGs for LABC were obtained from the dataset, among which 35 were up-regulated and 73 down-regulated. COL6A2, CRYAB, KRT16, MAP2, FSCN1, EMP1, COL3A1, and TUBB6 were identified to be significantly enriched in the EMT pathway ($P = .0057$) and cell growth and/or maintenance pathway ($P = .0042$) based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis in the up-regulated DEGs. Meanwhile, in the down-regulated DEGs, NRIP1, AR, FOXA1, TAT, TFF1, GREB1, IGF1R, BCL2, VAV3, HMGCS2, MYB, ERBB4, GATA3, TFF3, TOX3, and ESR1 were

Figure 1 Protein-Protein Interaction Network of DEGs. The Nodes Represent Proteins, and the Edges Indicate the Predicted functional Associations. The Line Thickness Indicates the Strength of Connections or Evidences

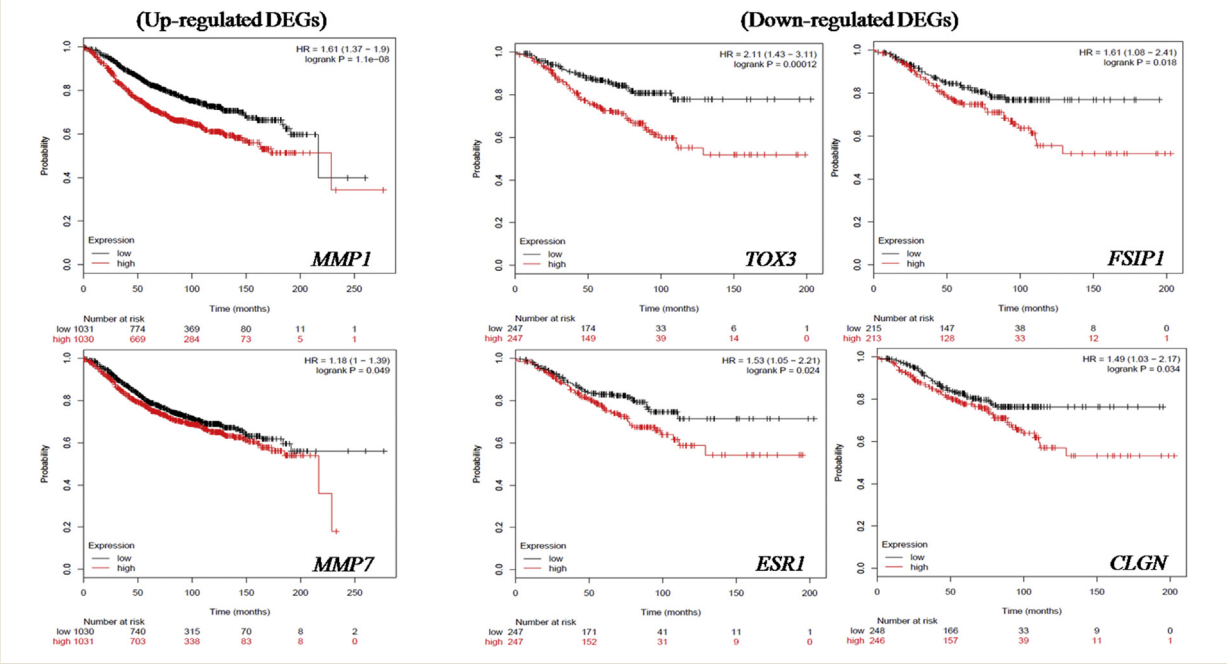


Abbreviation: DEGs = differentially expressed genes.

enriched in the FOXA transcription factor network ($P = 2.62E-06$) pathway, validated nuclear ER alpha network ($P = .00028$), ErbB receptor signaling network ($P = .0083$), AR ($P = .007$), and MET ($P = .0001$). These pathways are closely correlated with the progression, metastasis, and recurrence of breast cancer.

In the up-regulated DEGs, calcium-binding proteins S100A8, an important immunemodulatory factor that was correlated with lymph node metastasis, was considered to be a biomarker of poor prognosis in invasive ductal carcinoma of the breast.[26] S100A8 was also correlated to lower overall survival and worse outcome in patients with LABC.[27] In the study conducted by Bao et al, high expression and secretion of S100A8/A9 may be associated with the loss of ESR1 and GATA3 expression in breast cancer.[28] Interestingly, this phenomenon coincides with our results that ESR1 and

Figure 2 Kaplan-Meier Survival Analysis of MMP1, MMP7, TOX3, FSIP1, ESR1, and CLGN. The Survival Curve was Performed Using the Kaplan-Meier Estimator. The figure on the Left Represents the Survival Curve of Up-regulated DEGs; whereas the figure on the Right Represents the Survival Curve of down-regulated DEGs



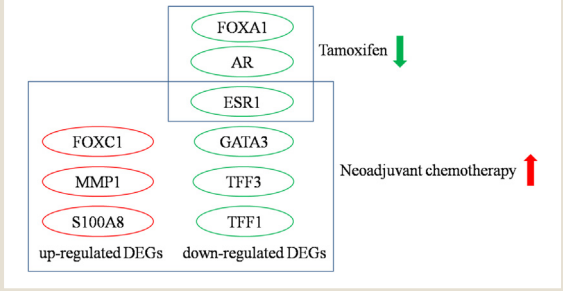
Abbreviations: DEGs = differentially Expressed Genes; HR = hazard ratio.

GATA3 were decreased by soy food intake. MMP1 and MMP7 were higher in the soy food group, and it has been reported that high expressions of MMP1 and MMP7 were correlated with worse overall survival and unfavorable relapse-free survival and were significant prognostic indicators for breast cancer.[29,30] Up-regulated DEGs of S100A8, MMP1, and MMP7 were hazardous factors for breast cancer induced by soy foods. On the other hand, Wang et al showed that increased expression of FOXC1 reduced the expression of ERα and cellular responses to tamoxifen in the MCF-7 cells.[31] Kolacinska et al found that high expression of FOXC1 means better pathologic response to neoadjuvant chemotherapy regardless of breast cancer subtypes.[32] Taken together, up-regulated DEGs of S100A8, MMP1, MMP7, and FOXC1 induced by soy foods were closely related to poor prognosis in patients with LABC.

In down-regulated DEGs, ERBB4 was related to the mammary gland alveolus development, cell proliferation, positive regulation of intracellular estrogen receptor signaling pathway, and negative regulation of apoptotic process. Bieche et al showed that lower ERBB4 expression meant better prognosis than ERBB4 over-expression in breast cancer.[33] Han et al has demonstrated that the expression levels of TOX3 were upregulated significantly in the T3 and T4 stages compared with the T1 stage.[34] And TOX3 was strongly associated with ER^+ breast cancers by GWAS.[35] Shan et al reported that overexpression of TOX3 was associated with reduced disease-free and metastasis-free survival rates.[36] These studies support that high expression of TOX3 is a risk factor for breast cancer. The expression of TOX3 after soy food treatment was significantly decreased compared with the placebo group ($P < .005$). In this respect, soy foods are a good diet for LABC.

FOXA1 was proved to be essential for cellular response to tamoxifen, even in those that have transitioned to tamoxifen resistance.[37] FOXA1 may also result in well-differentiated breast cancer and over-expressed ER.[38] Therefore, lower expression of FOXA1 induced by soy food intake might influence the effectiveness of tamoxifen. Moreover, Cochrane et al found that a high ratio of AR to ER ($\geq$ 2.0) indicated an over 4-fold increase of risk for failure when on tamoxifen.[39] Nevertheless, down-regulated DEGs showed that the decrease of ESR1 (−3.54 logFC) was higher than AR (−2.39 logFC), which suggested that the ratio would be bigger and might also reduce the curative effect of tamoxifen. Guarneri et al found that patients with lower ER pathway activity had high levels of chemotherapy sensitivity.[40] Gianni et al reported that combining ER status with ER-related genes was a novel and important method for predicting the response of a neoadjuvant chemotherapy and provided useful information for investigators to identify subgroups of patients who will either benefit or be resistant to neoadjuvant chemotherapy.[41] Microarray research has showed that S100A8/MMP1 were up-regulated, whereas ER, PR, GATA3, TFF1, and TFF3 were down-regulated in tumor biopsy from the patients with pathologic complete response. Chen et al suggested that such information might contribute to individualized cancer therapy.[42] In this study, the up-regulated genes of S1008A and MMP1 and the down-regulated genes (ERS1, TFF3, TFF1, GATA3) indicated that soy food treatment was likely to increase the efficacy of neoadjuvant chemotherapy. In these genes, TFF3 and TFF1, which have positive correlation with ER^+ breast cancer, were reported to stimulate migration and invasion of breast cancer cells.[43-45] Moreover, TFF3 and TFF1 were specific and sensitive predictive biomarkers of response to endocrine therapy, degree of response, and duration of response in unstratified patients with metastatic breast cancer.[46,47] And GATA3, which was strongly associated with ER^- and progesterone receptor-negative status,[6,48] was a good independent predictor of response to neoadjuvant chemotherapy at lower expression.[49] Guha et al suggested that soy foods consumed at levels comparable to those in Asian populations might reduce the risk of cancer recurrence in women receiving tamoxifen therapy and, moreover, appeared not to interfere with tamoxifen efficacy.[50] However, the present study revealed that soy food treatment probably lowers the curative effect of tamoxifen in LABC.

Figure 3 Low Expression of ERS1, FOXA1, and AR Tended to Weaken the Sensitivity of Tamoxifen; high Expression of FOXC1, S1008A, and MMP1 and low Expression of ERS1, TFF3, TFF1, and GATA3 Were Likely to Increase the Efficacy of Neoadjuvant Chemotherapy



Abbreviation: DEGs = differentially expressed genes.

Conclusion

In summary, we intended to identify DEGs with bioinformatics analysis to find the critical molecule change after soy food treatment in LABC in this study. In our study, a total of 108 DEGs were screened out. The up-regulated genes of MMP1 and MMP7 significantly reduced the overall survival time, whereas the down-regulated genes of TOX3, FSIP1, ESR1, and CLGN increased the overall survival time. In another important aspect, the expression patterns of FOXC1, S100A8/MMP1, ESR1, GATA3, TFF1, TFF3, FOXA1, and AR might be related to the efficacy improvement of neoadjuvant chemotherapy and the effectiveness reduction of tamoxifen after soy food treatment in LABC (Figure 3). Owing to small sample size, it still remains to be confirmed by further experimental studies to verify the results.

Clinical Practice Points

- Soy foods contain numerous biologically active compounds, such as genistein and isoflavones, which have estrogenic activity. Isoflavones have been reported to decrease breast cancer cell growth through ER-independent inhibition of tyrosine kinases and DNA topoisomerases, and dietary levels of genistein have also been reported to stimulate growth of estrogen-sensitive breast cancer cells through transactivation of the ER and

blocking the inhibitory effects of tamoxifen. However, the overall impact of soy foods in patients with LABC is still unclear.

- In this study, we found that soy foods could alter the ER-related gene profile in LABC dramatically. And, above all, the differentially expressed genes are strongly correlated to the sensitivity of tamoxifen and the curative effect of neoadjuvant chemotherapy. The result might provide a dietary reference for patients with LABC during drug therapy. Certainly, larger clinical trials are warranted to validate this association.

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Disclosure

The authors have stated that they have no conflicts of interest.

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