Bioinformatics Analysis of TIMP1, HK2 and IGFBP7 as Potential Biomarkers and Therapeutic Targets of Paclitaxel Resistance in Breast Cancer

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Abstract

Background: Paclitaxel is widely used as an adjuvant therapy in the treatment of breast cancer, yet its effectiveness decreases due to resistance problems. We conducted the present study to identify the potential paclitaxel resistance biomarkers and therapeutic targets in breast cancer employing bioinformatics approach.

Method: The present systematic bioinformatic study included a microarray data obtained from Gene Expression Omnibus database, which are respectively cell lines and tumor data from patients. We carried out Gene ontology, Kyoto Encyclopedia Genes, and Genome pathway enrichment analysis with The Database for Annotation, Visualization and Integrated. The protein-protein interaction network was analyzed with STRING-DB and visualized with Cytoscape. We confirmed of the reliability of the hub genes in paclitaxel sensitive and resistant breast cancer cells utilizing ONCOMINE. The prognostic value of the hub genes was evaluated using Kaplan-Meier survival curves.

Results: Gene ontology analysis revealed that differential expressed genes take part in cell adhesion, located in cellular component and paly a negative role in the regulation of reactive oxygen species. The protein-protein interaction network analysis, confirmed with ONCOMINE and Kaplan Meier survival, revealed three hub genes (*TIMP1*, *HK2*, and *IGFBP7*). Kyoto Encyclopedia Genes and Genome pathway enrichment analysis revealed the regulation of HIF-1 signaling pathway. Kaplan Meier survival plot showed that patients with high mRNA of *TIMP1*, *HK2*, and *IGFBP7* had significantly worse overall survival than those in the low expression level group.

Conclusion: *TIMP1*, *HK2*, and *IGFBP7* are not only biomarkers, but also potential targets to circumvent paclitaxel resistance in breast cancer.

Keywords: Breast cancer, Paclitaxel, Drug resistance, Bioinformatics, Biomarkers

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Introduction

Paclitaxel is widely used as a first-line chemotherapy for the treatment of breast cancer. However, high frequencies of recurrence and progression in treated patients indicate that metastatic breast cancer cells can become resistant against this drug.[1] Response rate to paclitaxel among breast cancer patients resides in a loose range of 10-60%.[2] Accordingly, understanding molecular mechanism and discovery of paclitaxel resistance is of great importance to achieve better efficacy of the treatment. In order to overcome paclitaxel resistance in breast cancer, a lot of studies have been conducted on biomarker using several types of breast cancer cells. Studies using MCF-7 luminal A breast cancer cells revealed several regulatory genes and biomarkers in paclitaxel resistance including *RDC1*, *IFI-30*, *FURIN*, *BCL2*, *S100P*, *CAV1* and *MC*,[3] *MDR1*,[4] *TRIP6, HSP27* and cathepsin D,[5] *ABCB1*,[6] and *miR-451* and *YWHAZ*[7] play a role in the mechanisms of paclitaxel resistance in MDA-MB 231 triple negative breast cancer cells. In addition, cellular senescence and cytoprotective autophagy are potential mechanisms of paclitaxel chemoresistance in the triple negative breast cancer.[8] A comprehensive cohort study on breast cancer patients demonstrated that multiple transcriptional fusions of *MDR1* is observed in paclitaxel resistant breast cancer cells.[9] Moreover, a recent bioinformatics study explored gene expression-based predictive markers for paclitaxel treatment in ER+ and ER- breast cancer.[2] Altogether, previous studies on paclitaxel-resistance biomarkers have been done using paclitaxel treated resistant cell line and patient data, whose results are very diverse; and thus, could not be employed for paclitaxel resistance cases in general. A review article showed that there are no valid practical biomarkers to predict the occurrence of paclitaxel resistance in breast cancer; and therefore, several biomarkers are needed to estimate paclitaxel chemoristance.[10] In this study, we used a bioinformatics approach to investigate the general biomarkers from a broad characteristics of samples utilizing both cell lines and patients' data. We also validated the expression of biomarker candidate with Oncomine database and the overall survival related to the level of expression of these genes. Thus, in this study, we found biomarker properties that can be used generally for diagnostic and prognostic of paclitaxel resistance in breast cancer (*TIMP1*, *HK2* and *IGFBP7*). Additionally, these three biomarkers could also be applied as drug target including gene therapy, monoclonal antibody, enzyme inhibitor, and therapeutic protein.

Material and Methods

Data collection and processing

The present systematic bioinformatics study included a microarray data obtained from Gene Expression Omnibus (GEO) database GSE12791[11] and GSE22796[12] (Table 1). We conducted the data processing with GEO2R, an online tool for GEO data analysis based on R programming language. DEGs between paclitaxel sensitive and resistant cells/tissues were screened. Adjusted *P* value <0.05 and IFCI >1.5 were used to select significant DEGs. We utilized Venny 2.1 to design a venn diagram in order to identify differentially expressed genes (DEGs) from two mRNA expression profile GSE12791 and GSE22796.[13]

Analysis of protein-protein interaction network and hub genes selection

Analysis of protein-protein interaction (PPI) network was constructed with STRING-DB v11.0[14] with confidence scores of >0.4 and was visualized with Cytoscape software.[15] Genes with a degree over 5, analyzed with CytoHubba plugin,[16] were selected as hub genes.

Gene ontology analysis, Kyoto encyclopedia genes, and genome (KEGG) pathway enrichment

Analysis of gene ontology (GO), Kyoto encyclopedia of genes, and genomes (KEGG) pathway enrichment were conducted with the database for annotation, visualization and integrated discovery (DAVID) v6.8,[17] and *P*<0.05 was selected as the cut-off value.

Table 1. Description of GSE datasets

Accession No.	Description	References
GSE12791	MDA-MB-231 cells (parental, n=4) were treated to regimen dose: 3-day treatment with 30 nM paclitaxel and followed by a 7-day recovery to generated paclitaxel resistant MDA-MB-231 cells (MDA-PR, n=4). The resistant cells were established within 8 cycles of such treatment (80 days).	Luo W, et al.[11]
GSE22796	Patients with residual invasive carcinoma following taxane-based chemotherapy (n=8) and the corresponding histologically benign breast tissue from 5 of the same 8 patients with post-therapy residual breast cancer was used as controls	Tan MH, et al.[12]

GSE: GEO (Gene expression omnibus) series

Validation of hub genes in paclitaxel resistant and sensitive breast cancer cells

We confirmed the reliability of the hub genes in paclitaxel sensitive and resistant breast cancer cells using ONCOMINE, a cancer microarray database and web-based data-mining platform.[18]

Kaplan Meier survival analysis

We evaluated the prognostic value of the hub genes employing Kaplan-Meier survival curves with log-rank test,[19] and *P*<0.05 was selected as the cut-off value.

Results

Identification of DEGs in paclitaxel resistant breast cancer

A total of 545 and 646 up-regulated genes were extracted from GSE12791 and GSE22796, respectively (Figure 1A). In addition, a total of 683 and 826 down-regulated genes were extracted from GSE12791 and GSE22796, respectively (Figure 1B). There were 85 genes consistently differentially expressed in the two datasets, consisting of 37 up-regulated and 48 down-regulated genes (Figure 1A-B).

Analysis of protein-protein interaction network and hub genes

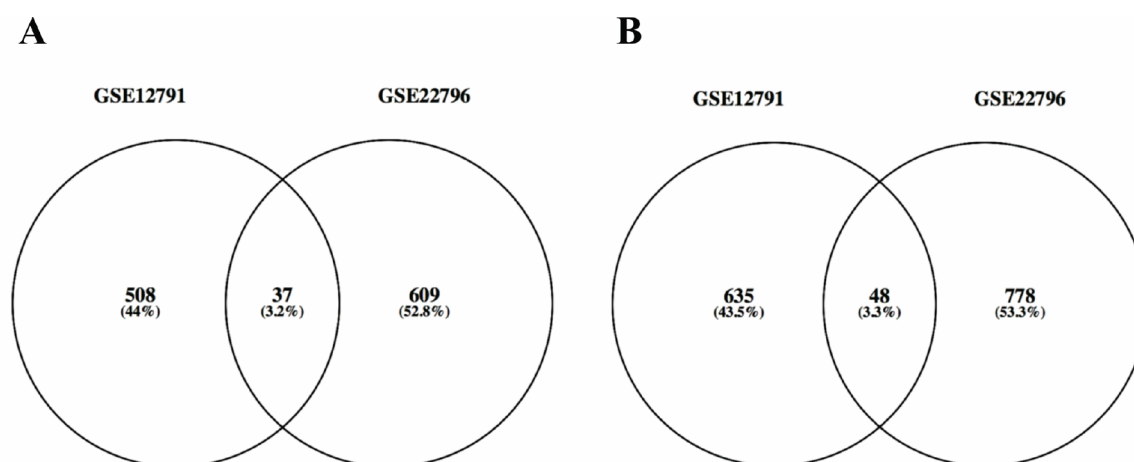A total of 85 genes were constructed to PPI

Figure 1. Venn diagram of (A) upregulated and (B) downregulated DEGs obtained from GSE12791 and GSE22796.
DEGs: Differentially expressed genes, GSE: GEO (Gene expression omnibus) series

Table 2. The hub genes identified in PPI Network as possessing > 5 degrees

Gene Symbol	Full Name	Description	Degrees
APP	Amyloid beta precursor protein	Up-regulated	12
TIMP1	TIMP metallopeptidase inhibitor 1	Up-regulated	11
CTGF	Connective tissue growth factor	Up-regulated	10
HK2	Hexokinase 2	Down-regulated	6
IGFBP7	Insulin like growth factor binding protein 7	Up-regulated	6

PPI : Protein-protein interaction

network complex containing 85 nodes and 82 edges, with an average node degree of 1.93 (Figure 2A). The five genes with degrees over 5 were identified as hub genes (*APP*, *TIMP1*, *CTGF*, *HK2* and *IGFBP7*) (Figure 2B, Table 2).

Gene ontology analysis and KEGG pathway enrichment of the hub genes

Gene ontology analysis of the hub genes illustrated that the hub genes regulate the biological process of regulation of cell growth and cell adhesion, located in extracellular 5 space, proteinaceous extracellular matrix, extracellular exosome, and golgi apparatus. They were also found to take part in the molecular function of heparin binding (Table 3). Moreover, KEGG pathway enrichment analysis revealed the regulation of HIF-1 signaling pathway by the hub genes (Table 4).
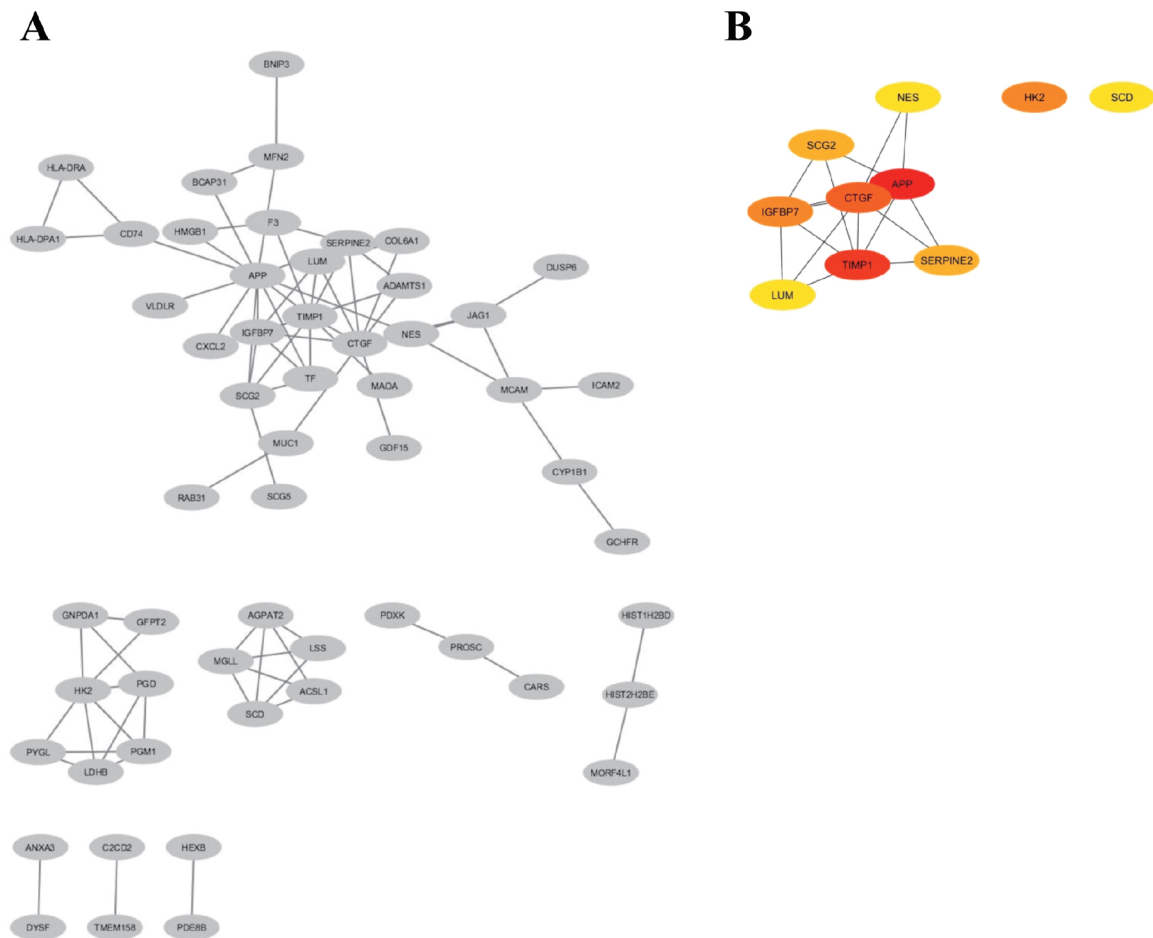

Figure 2. (A). Protein-protein interaction (PPI) network complex of DEGs, as analyzed with Cytoscape, (B) one cluster with the highest degree score, as analyzed with Cytohubba.
DEGs: Differentially expressed genes

Table 3. The enriched gene ontology terms of the hub genes

Category	Term	Count	*P* Value	Genes
Molecular function	GO:0008201~heparin binding	2	0.014750452.	*APP, CTGF*
Cellular component	GO:0005615~extracellular space	3	0.009849658	*APP, IGFBP7, TIMP1*
Cellular component	GO:0005578~proteinaceous extracellular matrix	2	0.034513946	*CTGF, TIMP1*
Cellular component	GO:0070062~extracellular exosome	3	0.061069157	*APP, IGFBP7, TIMP1*
Cellular component	GO:0005794~Golgi apparatus	2	0.098126309	*APP, CTGF*
Biological Process	GO:0001558~regulation of cell growth	2	0.006802698	*CTGF, IGFBP7*
Biological Process	GO:0007155~cell adhesion	2	0.033942964	*CTGF, IGFBP7*

GO: Gene ontology, *APP*: Amyloid beta precursor protein, *TIMP1*:TIMP metallopeptidase inhibitor 1, *CTGF*: Connective tissue growth factor, *IGFBP7*: Insulin like growth factor binding protein 7

Validation of hub genes in paclitaxel resistant and sensitive breast cancer cells

We utilized Oncomine to confirm the reliability of the hub genes in paclitaxel sensitivity. A study by Lee et al., using cell lines, showed an up-regulation of APP, *TIMP1*, *CCN2*, and *IGFBP7* (Figure 3) in paclitaxel resistant cells. A study by Gyorffy, using cell lines, indicated a down-regulation of *HK2* in paclitaxel resistant cells (Figure 3).

Kaplan Meier survival analysis

We obtained Kaplan Meier plot for overall survival of breast cancer patients according to the low and high expression levels of each gene.
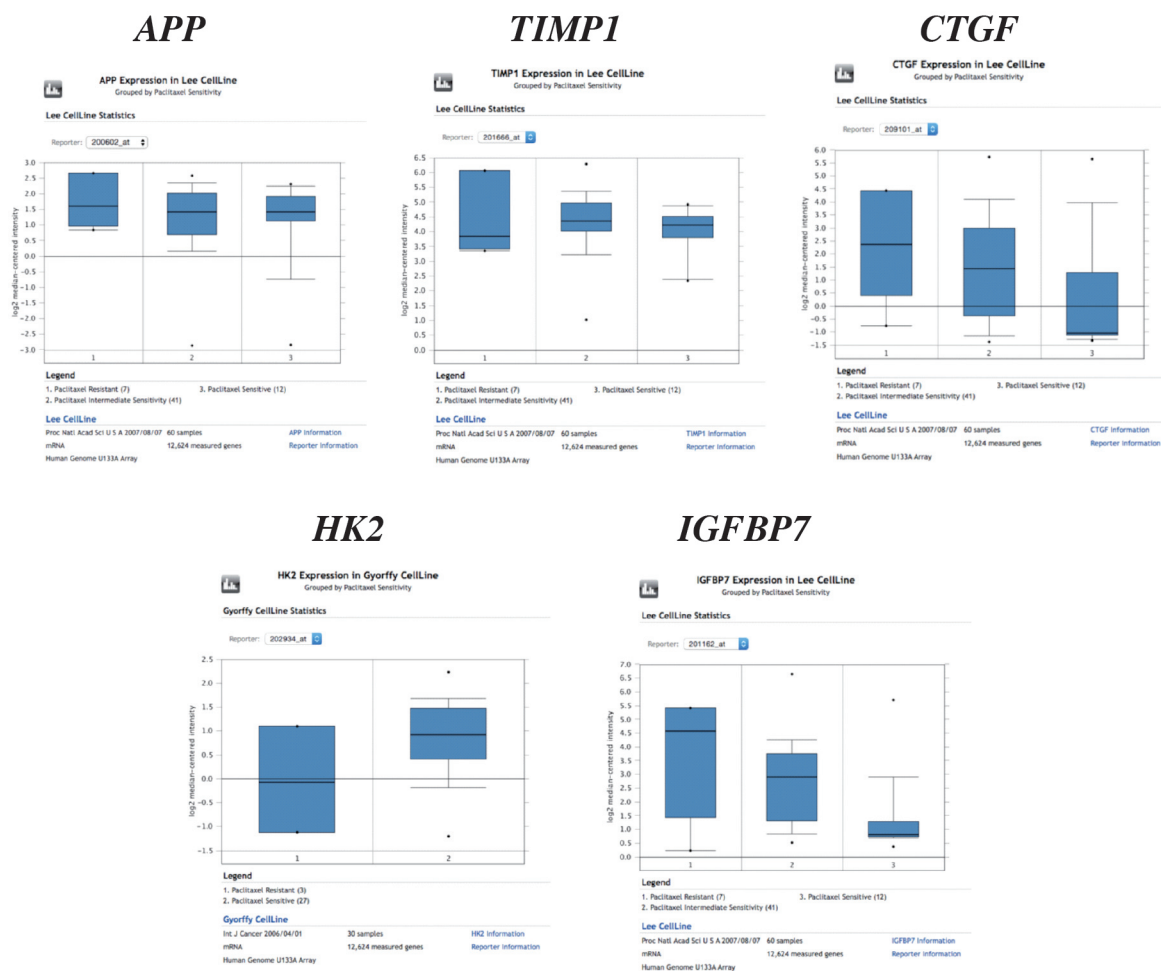

Figure 3. Expression of *APP*, *TIMP1*, *CTGF*, *HK2*, and *IGFBP7* in paclitaxel-resistant and sensitive breast cancer cells, as analyzed with Oncomine.

Table 4. KEGG pathway of the hub genes

Pathway	Count	*P* Value	Genes
HIF-1 signaling pathway	2	0.043364152	*HK2, TIMP1*

KEGG: Kyoto encyclopedia genes and genome, HIF-1: Hypoxia-inducible factor 1, *HK2*: Hexokinase-2, *TIMP1*: Metalloproteinase inhibitor 1

The results revealed that patients with high mRNA level of *TIMP1*, *HK2* and *IGFBP7* had significantly worse overall survival compared with those in the low expression level group, with *P*= 0.041, *P*=4.4e-6 and *P*=0.026, respectively (Figure 4).

Discussion

This present study identified the candidates for biomarker of paclitaxel resistance in breast cancer using bioinformatics approaches. We expected to obtain chemoresistance markers from various types of breast cancer as well as molecular targets to overcome paclitaxel resistance in breast cancer. Based on the PPI network complex, ONCOMINE, and Kaplan meier survival analysis, three genes were selected as the potential biomarkers and therapeutic targets candidate of paclitaxel resistance (*TIMP1*, *HK2*, and *IGFBP7*). *TIMP1* encodes TIMP metallopeptidase inhibitor 1,[20] the key regulator of the metalloproteinases, which degrades the extracellular matrix and sheds cell surface molecules.[21] The overexpression of *TIMP1* is attributed to anthracyclin resistance in breast cancer.[22] The high expression of *TIMP1* in serum is assigned to progression and worse survival in gastric cancer patients.[23] Several studies have demonstrated targeting *TIMP1*, including

gene therapy using adenoviral vector24 and TIMP1 blocking antibody in human dermal microvascular endothelial cells.[25] Therefore, the development of targeted therapy against *TIMP1* needs to be further explored to overcome paclitaxel resistance in breast cancer. *HK2* encodes hexokinase 2, a key enzyme and the first rate-limiting enzyme of glycolysis.[26] Cancer cells show deregulation of cellular energy from oxidative phosphorylation to aerobic glycolysis known as Warburg effect.[27] *HK2* is overexpressed in many human cancers and correlates with chemoresistance and poor prognosis of brain metastasis of breast cancer patients[28] and neuroblastoma patients.[29] *HK2* also promotes ovarian cancer cells to cisplatin[30] and paclitaxel.[31] In addition, paclitaxel resistance in breast cancer is regulated by PIM2-mediated phosphorylation of hexokinase 2.[32] Accordingly, *HK2* is an important target for overcoming paclitaxel resistance in breast cancer. Some studies have developed HK2-targeted therapies using HK2 inhibitors, for instance metformin, 2-Deoxyglucose, and 3-Bromopyruvate in colon, breast, and hepatocellular carcinoma.[33,34] Benserazide, a dopadecarboxylase inhibitor, suppresses tumor growth by targeting HK2.[34] Ketoconazole and posaconazole selectively
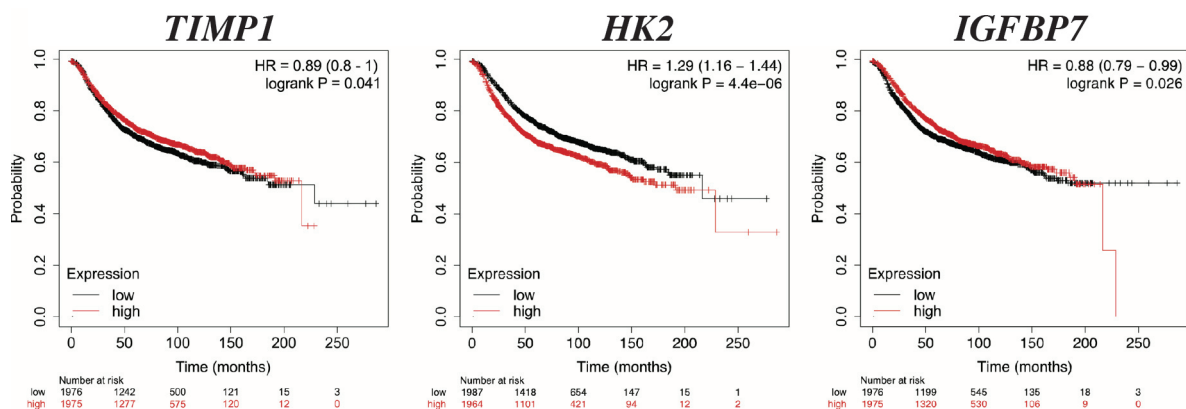

Figure 4. Overall survival of *TIMP1*, *HK2*, and *IGFBP7* across breast cancer samples, as analyzed with KM plotter.

eradicates *HK2*-expressing glioblastoma cells.[35] Accordingly, further study is needed on the development of the above-mentioned compounds in order to overcome paclitaxel chemoresistance in breast cancer patients. *IGFBP7* encodes Insulin-like growth factor (IGF) binding protein 7, a member of IGFBP, which protects IGFs from degradation in circulation by forming a high affinity complex.[36] Binding of IGFs to IGFBPs might inhibit the interaction between the IGFs and their receptor, IGFRs.[37] Regarding the role of *IGFBP7* in chemoresistance, the overexpression of *IGFBP7* is attributed to the resistance against vincristine, etoposide, and asparaginase and negative outcome in Jurkat adult T-cell acute lymphoblastic leukemia cells.[38] In addition, the overexpression of *IGFBP7* or administration of recombinant human *IGFBP* (rhIGFBP7) resulted in an increased doxorubicin and cytarabine sensitivity of primary acute myeloid leukemia cells.[39] Accordingly, *IGFBP7* could be utilized to increase the sensitivity of breast cancer cells to paclitaxel. KEGG pathway enrichment analysis revealed the regulation of HIF-1 signaling pathway by *TIMP1* and *HK2*. Hypoxia-inducible factor-1alpha (HIF-1alpha), a transcription factor induced by low oxygen concentration and overexpression of malignant solid tumors,[40] promotes the overexpression and activity of several glycolytic transporters, such as *GLUT1*, *GLUT3*, and enzymes, for instance *HK1*, *HK2*, and *PFK-L*.[40] In addition, the activation of HIF-1 signaling pathway was found to promote chemoresistance in MDA-MB-231 breast cancer cells.[41] Previous studies have demonstrated the axis between *TIMP1*, *HK2*, and HIF signaling pathways. The combinatorial treatment of polydatin and 2-deoxy-d-glucose in breast cancer cells enhances cell death by targeting the ROS/PI3K/AKT/HIF-1α/*HK2* axis.[42] *TIMP1* expression is regulated by HIF1 in vascularization[43] and liver metastasis.[44] Moreover, the treatment of recombinant human rhTIMP-1 promotes cell survival and increases mRNA level of HIF1 α in acute myeloid leukemia cells.[45] In the signaling regulation of thyroid carcinogenesis, knockdown of *STAT3* increases the expression of HIF α and the down-regulation

of *IGFBP7*.[46] However, the relations between HIF and *IGFBP7* remain elusive and need to be further clarified. Accordingly, further studies could be suggested on HIF-1 signaling in paclitaxel resistance in breast cancer.

Paclitaxel resistance in luminal A and triple negative breast cancer are associated with switching the mechanism from apoptotic to autophagic cell death,[47] cellular senescence and cytoprotective autophagy.[8] The inhibitors of HIF-1 may impair the metabolic flexibility of cancer cells and make them more sensitive to anticancer drugs.[48] This work shed light to the fact that *HK2*, *IGFBP7*, and *TIMP1* are biomarker candidates of paclitaxel resistance, which involve in the HIF signaling. The increased expression of *TIMP1* is observed in senescence fibroblast.[49] *HK2* regulates autophagy induced by glucose starvation.[50] IGFBP 7 promotes senescence in mesenchymal stem cells.[51,52] In sum, further study is required on senescence and autophagy mechanism related to *TIMP1*, *HK2*, *IGFBP7*, and paclitaxel resistance. The current research had several limitations, including the mRNA data used for the PPI network. This might give different results since the expression of mRNA is not always correlated to the protein level. In this study, we also employed bioinformatics approaches; therefore, further studies are needed to validate the biomarker as well as the molecular target in order to overcome paclitaxel resistance in breast cancer.

Conclusion

In conclusion, the present paper not only explored potential targets to circumvent breast cancer resistance to paclitaxel, but also provided novel approaches to cancer therapeutics in terms of overcoming paclitaxel resistance in breast cancer. HIF-1 signaling pathway plays a pivotal role in breast cancer resistance to paclitaxel. More importantly, *TIMP1*, *HK2*, and *IGFBP7* are not only biomarker candidates for paclitaxel resistance, but also potential targets to circumvent paclitaxel resistance in breast cancer patients.

Conflict of Interest

None declared.

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