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ORIGINAL ARTICLE

Therapeutic Potential of *PLK1, KIF4A, CDCA5, UBE2C, CDT1, SKA3, AURKB,* and *PTTG1* Genes in Triple-Negative Breast Cancer: Correlating Their Expression with Sensitivity to GSK 461364 and IKK 16 Drugs

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Abstract

The treatment of triple-negative breast cancer (TNBC) has been associated with challenges due to the lack of expression of ER, PR, and HER2 receptors in tumor cells. This study aimed to identify genes with potential therapeutic targets in TNBC. Data from the cancer genome atlas regarding breast cancer (BC) were downloaded. After initial preprocessing, cancer samples were categorized into four groups: TNBC, HER2-positive, luminal A, and luminal B. Gene expression differences between these groups were calculated, focusing on genes that showed differential expression in TNBC. A protein–protein interaction network was conducted to identify hub genes among the candidate genes related to TNBC. The protein expression of candidate genes was assessed using immunohistochemistry data from the human protein atlas. Drug resistance and sensitivity associated with hub genes were identified using data from PharmacoDB. TNBC samples and the RT-qPCR method were used to confirm the results. Our findings revealed that eight genes, namely *PLK1, KIF4A, CDCA5, UBE2C, CDT1, SKA3, AURKB, and PTTG1*, had significant upregulation at the RNA level in TNBC subgroup compared to other subgroups and could be considered hub genes in TNBC. Compared to other subgroups, their expression level in TNBC samples had high sensitivity and specificity. RT-qPCR results also demonstrated a significant increase in levels of *SKA3* and *PTTG1* in the TNBC compared to healthy tissue and other subgroups. The protein expression of these genes was notably high in some BC samples. PharmacoDB data showed that some candidate genes were closely linked to drug sensitivity of GSK 461364 and IKK 16. The results of this study showed a significant increase in the expression level of *PLK1, KIF4A, CDCA5, UBE2C, CDT1, SKA3, AURKB,* and *PTTG1* in TNBC compared to other BC subgroups. These genes show considerable promise as therapeutic targets for the TNBC subgroup.

Extended author information available on the last page of the article

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Keywords Breast cancer · Gene expression · Drug sensitivity · Real time PCR · Triple-negative

Abbreviations

BC	Breast cancer
ER	Estrogen receptor
PR	Progesterone receptor
HER2	Human epidermal growth factor receptor 2
TNBC	Triple-negative breast cancer
TCGA	The cancer genome atlas
PPI	Protein–protein interaction
ROC	Receiver operating characteristic
AUC	Area under the curve
K–M	Kaplan–Meier
FDR	False discovery rate

Introduction

Breast cancer (BC) was the most diagnosed cancer among American women in 2020. It is the second-leading cause of cancer-related death in women, after lung cancer (Giaquinto et al. 2022). Similar to other common cancers, BC exhibits high molecular heterogeneity, with both environmental and genetic factors contributing to its risk (Polyak 2011). While the mortality rates among affected individuals of BC have significantly decreased in recent years, BC treatment remains a substantial challenge with uncertainties. Consequently, identifying the molecular mechanisms involved is crucial for discovering new therapeutic targets.

BC is classified into four subtypes based on the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). These subtypes include HER2+ (positive for HER2 and negative for ER and PR), luminal A (positive for ER and/or PR and negative for HER2), luminal B (positive for ER and/or PR and HER2), and triple-negative breast cancer (TNBC) (Tang et al. 2016). Patients in the TNBC subgroup lack expression of HER2, ER, and PR receptors. Studies indicate that TNBC subtype exhibits poorer treatment responses compared to other subtypes due to the absence of the mentioned receptors (Dent et al. 2007). Moreover, this subtype has a poorer prognosis, comprising nearly 15% of invasive BC (Brouckaert et al. 2012). TNBC tumor cells lack HER2, ER, and PR receptors; hence, there is no particular effective treatment for them, and chemotherapy's efficacy is still very limited (Garrido-Castro et al. 2019). Several targeted therapies have been developed for TNBC based on its specific molecules and pathways. These include inhibitors for PI3K/AKT/mTOR, EGFR, Notch, and poly ADP-ribose polymerase. Also, immune checkpoint inhibitors like pembrolizumab, atezolizumab, and durvalumab are widely studied in clinics (Zhu et al. 2023). However, targeted and effective treatment for TNBC has

not yet been reported. Therefore, identifying key genes involved in the development of TNBC is crucial for discovering new therapeutic targets.

While many studies have explored and identified genes linked to TNBC, there has been less focus on comprehensive and comparative analyses of gene expression between TNBC and other subtypes. This study aimed to identify genes in the TNBC subgroup whose expression differs significantly from HER2+, luminal A, luminal B, and healthy subtypes. To achieve this, we initially identified genes using data from the cancer genome atlas (TCGA) data. Protein–protein interaction (PPI) networks were utilized to identify hub genes associated with the TNBC subgroup. Moreover, the expression of candidate genes at the protein level was examined using the human protein atlas database. The relationship between the expression of candidate genes and drug resistance or sensitivity was investigated based on data from the PharmacoDB database. Finally, to confirm the obtained results, the expression levels of two genes among the candidate genes in TNBC samples compared to other subtypes and healthy tissue were evaluated using RT-qPCR.

Materials and Methods

Data Mining and Initial Preprocessing

For the investigation of the transcriptome of various subgroups of BC, data from the TCGA database were utilized. Initially, BC data were downloaded using the TCGAbiolinks package (Colaprico et al. 2016). Subsequently, genes with zero or near-zero expression, based on a criterion of CPM (counts per million) less than 10 in 70% of the samples, were excluded (Liu et al. 2021). Normalization of the data was performed using the TMM (trimmed mean of M values) method, followed by log2 transformation (Robinson and Oshlack 2010). Transcriptome information for 1109 malignant samples and 113 healthy samples was included in the dataset. In addition, clinical data were used to determine the status of each cancer sample in terms of ER, PR, and HER2 receptors in this database. Furthermore, the study GSE30931 was employed to explore gene expression changes in response to the drug Bortezomib in a BC cell line. Raw data from this study were downloaded, and initial preprocessing steps such as background correction, data normalization, and log2 transformation were conducted (Smyth 2005). The resulting expression matrix was used for differential expression analyses. The BC cell line known as MCF7 was treated with boratezamib in GSE30931.

Differential Expression Analysis

The BC samples obtained from the TCGA database were classified into four groups based on receptor status: HER2+, luminal A, luminal B, and TNBC. After clinical data assessments, it was determined that out of 1109 cancer samples from the TCGA, 42 samples were in the HER2+ group, 456 samples were in the luminal A group, 155 samples were in the luminal B group, and 118 samples were in the

TNBC group. The remaining cancer samples with ambiguous receptor statuses were excluded from the analysis. To identify genes with significant expression differences among these subgroups, differential expression analysis was performed using a linear model approach (Law et al. 2014). Additionally, data from each subgroup was compared to normal samples. In the case of GSE30931, differential expression between the Bortezomib-treated samples and the control group was calculated.

The PPI Network, Enrichment, and Sensitivity and Specificity Analysis

The enrichr tool (https://maayanlab.cloud/Enrichr/) and data from the msigDB database were employed to identify pathways associated with the selected genes for each subgroup. The stringDB (https://string-db.org/) database was utilized to identify hub genes related to the TNBC subgroup. Genes with higher or lower expression in TNBC and those related to patient survival were considered. Additionally, hub genes were selected based on a criterion of degree higher than the average, and the results were visualized using Cytoscape (V10). Receiver operating characteristic (ROC) curves were utilized to investigate the sensitivity and specificity of candidate genes, with the area under the curve (AUC) considered as the measure.

Prognosis and Survival Rate

The association between candidate gene expression in TNBC and patient survival rates was investigated using normalized expression matrices and clinical data from TCGA. The preprocessing of clinical data involved removing samples with survival statuses of 0, 1, and NA. Gene expression data in the normalized expression matrix was transformed into Z-score values and integrated with preprocessed clinical data. Finally, Cox regression analysis was used to examine the relationship between the expression of candidate genes and patient outcomes. Kaplan–Meier (K–M) curves were employed to validate the obtained results, with the median expression of candidate genes in cancer samples as a cut-off to classify samples into high and low groups.

Drug Resistance and Sensitivities

We studied candidate genes in the TNBC group for their potential association with drug resistance and susceptibility using data from PharmacoDB (Smirnov et al. 2018). Two datasets of BC cell lines, namely GRAY (breast cancer screen by Dr. Joe Gray's lab) and UHNBreast (university health network breast cancer screen), were utilized for this purpose. Analyses for these two pharmacosets were conducted using the PharmacoGX package (Smirnov et al. 2016). Only results with degrees of freedom greater than 10 were considered for assessment. Correlation coefficients greater than 0.4 and FDRs less than 0.01 were considered.

The Method of Selecting Important Genes as Therapeutic Targets in TNBC

The following criteria were considered to select important genes as therapeutic targets in TNBC: (1) significance of differential expression in TNBC compared to other breast cancer subtypes; (2) preferential differential expression restricted to the TNBC subgroup; (3) the correlation of expression changes with patient mortality rates; (4) detection of protein expression of these candidate genes in BC samples; and (5) recognition of these genes as hub nodes in PPI network analysis.

Collection of Samples

This study utilized a total of 65 BC samples and 20 healthy samples. A summary of the sample information is provided in Table 1. All samples were obtained from the Iranian Tumor Bank, and the cancerous samples were confirmed by a pathologist. All bioethical considerations were thoroughly reviewed and approved by the review board of Emam Khomeini Hospital, following the criteria set by the Ministry of Health and Medical Education of Iran. Moreover, written consent forms were obtained from all participating candidates. All samples were preserved in liquid nitrogen until their use.

RNA Extraction, cDNA Synthesis, Primer Design and RT-qPCR

RNA extraction for each sample was performed using the Trizol (Genius Gene, Iran) method following the manufacturer's guidelines. Before extraction, samples were

Table 1 Summary of clinical information of BC patients participating in this study

Subgroups	Number of samples	Stage (Number)
HER2+	15	Stage I (3)
		Stage II (7)
		Stage III (3)
		Stage IV (2)
Luminal A	15	Stage I (4)
		Stage II (10)
		Stage III (1)
		Stage IV (0)
Luminal B	15	Stage I (5)
		Stage II (9)
		Stage III (1)
		Stage IV (0)
TNBC	20	Stage I (4)
		Stage II (8)
		Stage III (5)
		Stage IV (3)
Healthy	20	–

washed several times with PBS to remove necrotic cells and tissue debris. DNase I (Sinaclon, Iran) treatment was employed to remove DNA contamination. Subsequently, cDNA synthesis (Takara, Japan) was conducted using oligodT and random hexamer primers according to the manufacturer's protocol. Specific primers for *SKA3* (F: 5′-CGTACGCCTGGAGTCCTTC-3′, R: 5′-TGGATAATCTTCAAAGTC GCTTTCC-3′) and *PTTG1* (F: 5′-TGGGGTCTGGACCTTCAATC-3′, R: 5′-GGC ATCGAACGTTTTGCCA-3′) were designed using the Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast). In primer design, we ensured that the primers bind specifically at the exon-exon junction to prevent unintended DNA amplification during RT-qPCR. The gene expression levels of *SKA3* and *PTTG1* in the study samples were assessed in triplicate using the designed primers and the SYBR Green (Genius Gene, Iran) method. The expression level of the B-actin gene was used as an internal control for data normalization.

Statistics and Software

Using R (v4.3.2), we conducted initial preprocessing steps on the raw TCGA data, utilizing the latest updates of the mentioned packages. The differential expression between groups was assessed using a linear model, with a threshold set at FDR < 0.01. Significance for RT-qPCR results was evaluated using a one-way ANOVA test. To assess significance in survival-related tests, the log-rank method was employed. All figures and plots were generated using GraphPad Prism software (v 8). To investigate the sensitivity and specificity of candidate genes, their expression levels in TNBC samples compared to other subgroups were analyzed through the ROC curve.

Results

Distinct Expression Alterations in Different Subgroups of BC

In the first stage, we used the TCGA-BC data to identify genes that show differential expression in TNBC compared to other subgroups. Results for the HER2+ subgroup revealed a total of 263 genes with logFC > 1 and FDR < 0.01, indicating significantly increased expression compared to other subgroups, including luminal A, luminal B, and TNBC (Fig. 1A). For the luminal A subgroup, 187 genes were upregulated in comparison to other subgroups (Fig. 1B). Furthermore, results for the luminal B and TNBC subgroups indicated 25 and 781 genes, respectively, were significantly upregulated compared to other subgroups (Fig. 1C, D). Conversely, downregulated genes were also examined. Our analysis identified 288 genes with logFC < − 1 and FDR < 0.01 that exhibited decreased expression in the HER2+ samples compared to other subgroups (Fig. 1A). Furthermore, significant downregulation was found for 778 genes in the TNBC subgroup, 12 genes in the luminal B subgroup, and 95 genes in the luminal A subgroup (Fig. 1B–D).

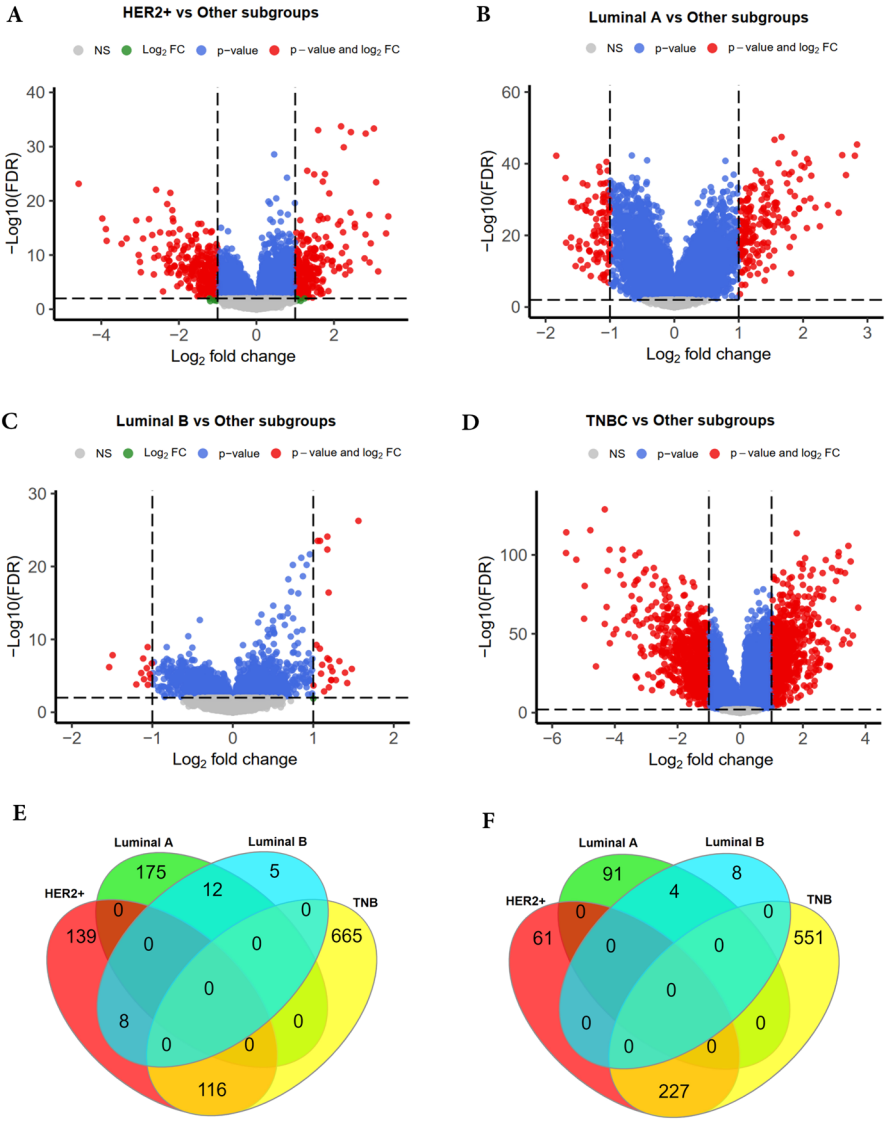

Fig. 1 Significant variations in gene expression profiles in different breast cancer subgroups. **A–D** Volcano plots illustrate the differential expression of genes within each BC subgroup compared to other subgroups. Genes were selected based on the criteria of |logFC|> 1 and FDR < 0.01. **E–F** A Venn diagram is shown to identify overlapping and specific genes in each subgroup

Venn diagrams were used to examine the intersection of upregulated and downregulated genes for each subgroup, aiming to identify genes with greater specificity for each. As depicted in Fig. 1E, among the previously upregulated genes, 139 were specific to the HER2+ subgroup, 175 to the luminal A subgroup, 5 to the luminal B subgroup, and 665 to the TNBC subgroup, with no overlap with other subgroups.

Additionally, downregulated genes included 61 genes for HER2+, 91 for luminal A, 8 for luminal B, and 551 for TNBC subgroups, showing distinct subgroup-specific expression patterns (Fig. 1F). Interestingly, a significant overlap was observed between the genes identified for luminal A and luminal B subtypes. (Fig. 1E, F). These findings suggest differential gene expression behavior across subgroups in BC. Moreover, these distinct gene expression profiles among subgroups may influence patient survival rates and treatment responses. The genes specifically identified through Venn diagrams were considered candidate genes for further analysis.

Involvement of TNBC-Related Genes in Cell Proliferation Pathways

The pathways related to the specific genes of each subgroup were examined. Results for upregulated genes related to the HER2+ subgroup indicated their involvement in pathways such as ABC transporters, KRAS signaling, and glycolysis (Fig. 2A, FDR < 0.05). Pathway analysis for upregulated genes associated with luminal A

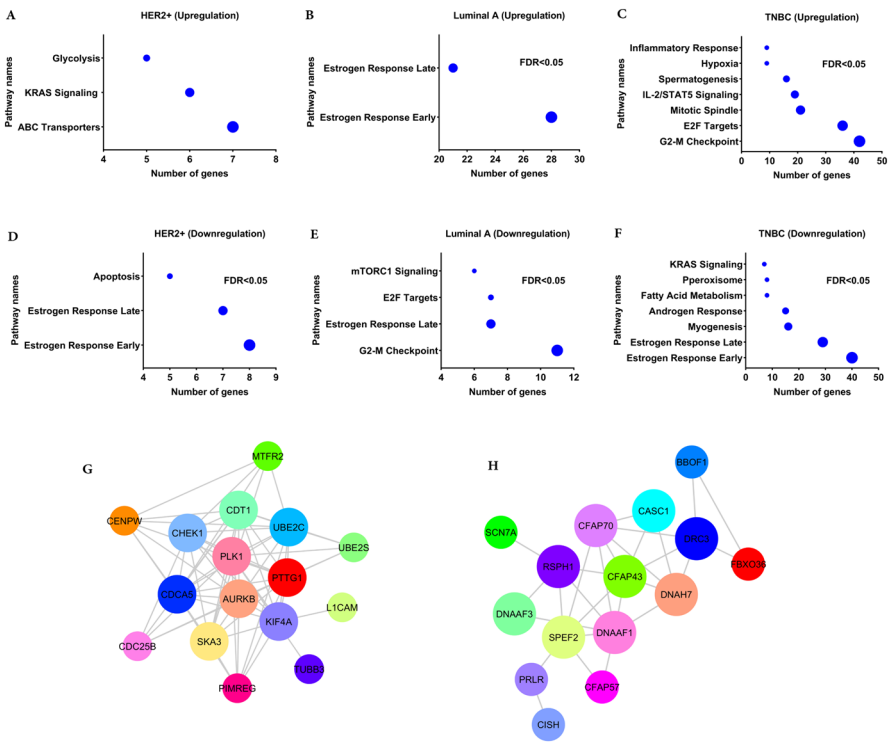

Fig. 2 Association of genes related to TNBC with cell proliferation, hypoxia, and inflammation pathways. **A**–**F** Enrichment results for genes associated with each subgroup (both upregulated and downregulated genes) are presented. **G** The PPI network is shown for all upregulated genes associated with poor prognosis in the TNBC subgroup. Data from the STRING database was used to construct the network, focusing on hub genes identified by their degree of connectivity. **H** The PPI network is presented for downregulated genes linked to a good prognosis in the TNBC subgroup

revealed their significant role in pathways related to the estrogen response (Fig. 2B, FDR < 0.05). In the TNBC subgroup, upregulated genes were significantly involved in pathways related to cell proliferation (E2F targets, G2-M checkpoint, mitotic spindle), inflammation, and hypoxia (Fig. 2C, FDR < 0.05). These findings suggest that genes related to malignancy processes, such as cell proliferation and inflammation, are more highly expressed in the TNBC subgroup compared to other subgroups.

For downregulated genes in HER2+ subgroup, pathways related to estrogen response and apoptosis were identified (Fig. 2D, FDR < 0.05). In the luminal A subgroup, downregulated genes were associated with pathways involving cell proliferation (E2F targets, G2-M checkpoint) and mTORC1 (Fig. 2E, FDR < 0.05). Pathways like estrogen response, fatty acid metabolism, KRAS, and peroxisome were identified for downregulated genes associated with the TNBC subgroup (Fig. 2F, FDR < 0.05). These findings imply that distinct pathways may be active for each subgroup associated with BC. No significant pathways were identified for the upregulated or downregulated genes associated with the luminal B subgroup.

The Correlation Between the Expression of Many Genes Specific to TNBC and Patients' Survival Rates

We focused on the genes identified for the TNBC subgroup. Initially, the correlation between the expression of 665 upregulated genes in the TNBC subtype and patient survival rates was analyzed using TCGA clinical data. Cox regression analysis identified 61 of the 665 upregulated genes as significantly associated with poor prognosis, as detailed in Table S1 (HR > 1 and log-rank < 0.05). PPI network analysis for these 61 genes identified *PLK1, KIF4A, CDCA5, CHEK1, UBE2C, CDT1, SKA3, AURKB*, and *PTTG1* as potential hub genes associated with TNBC (Fig. 2G). Among 551 downregulated genes related to the TNBC subtype, our results demonstrated that the expression of 72 genes was associated with a favorable prognosis (Table S1, HR < 1 and log-rank < 0.05). PPI network analysis for these 72 downregulated genes associated with a good prognosis in the TNBC subtype revealed *DNAAF1, CFAP70, RSPH1, DRC3, CFAP43, SPEF2,* and *DNAH7* as potential hub genes (Fig. 2H).

To validate the obtained results, K–M curve analysis was employed. The results showed that higher expression of *PLK1, KIF4A, CDCA5, CHEK1, UBE2C, CDT1, SKA3, AURKB,* and *PTTG1* was associated with higher mortality rates (Fig. 3, log-rank < 0.05). Increased expression of *DNAAF1, CFAP70, RSPH1, DRC3, SPEF2*, and *DNAH7* was associated with decreased patient mortality (Fig. 4, log-rank < 0.05). K-M analysis for *CFAP43* did not yield significant results, and this gene was excluded from further analysis. These findings suggest that 15 genes in the TNBC subtype, including *PLK1, KIF4A, CDCA5, CHEK1, UBE2C, CDT1, SKA3, AURKB, PTTG1, DNAAF1, CFAP70, RSPH1, DRC3, SPEF2*, and *DNAH7*, display notable differences in expression compared to other subtypes. These genes also show correlations with patients' mortality rates and may function as hub genes within the TNBC subtype.

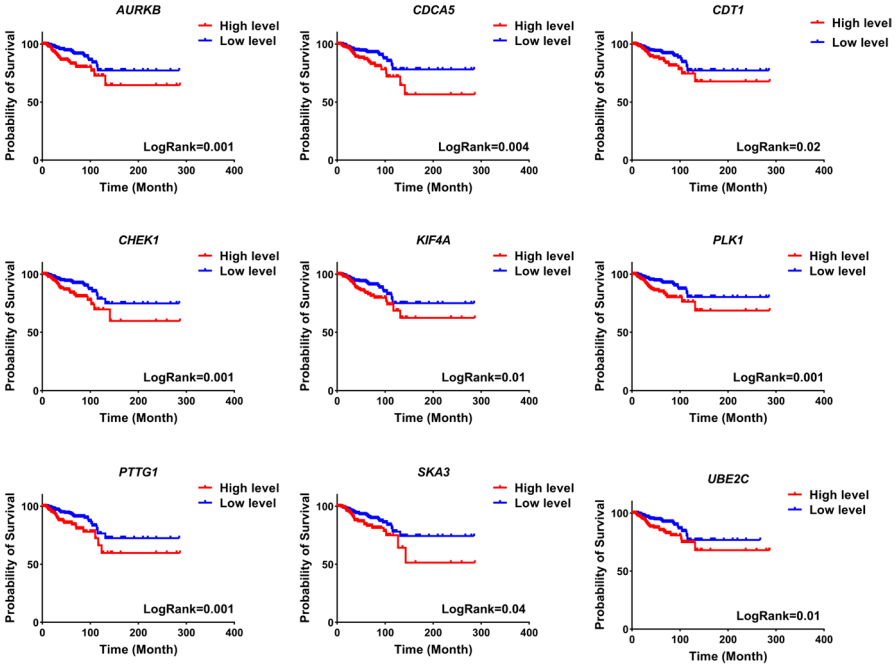

Fig. 3 The correlation between hub genes associated with the TNBC subgroup and increased mortality rates. The K-M curve is plotted for all identified hub genes within the TNBC subgroup, highlighting their expression correlation with patient mortality rates

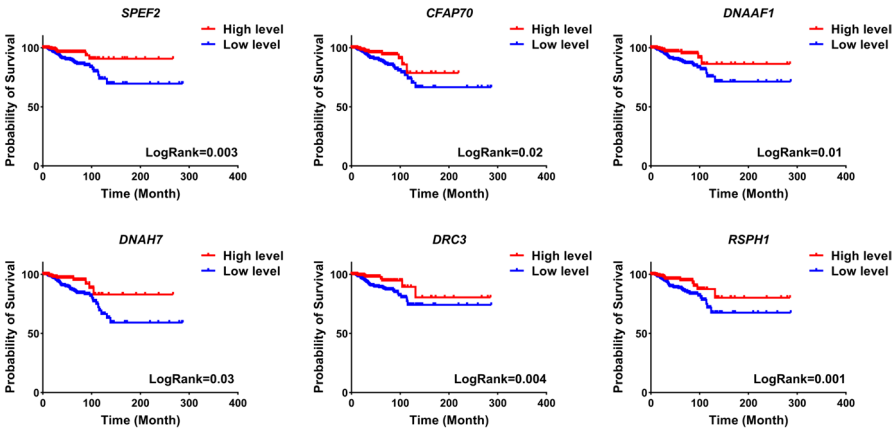

Fig. 4 Decreased expression of genes related to TNBC is associated with a favorable prognosis for patients. The connection between the downregulated expression of hub genes linked to TNBC and the mortality rates of patients is depicted based on clinical data obtained from TCGA

Differential Expression of Hub Genes Identified in TNBC Compared To Normal: Correlating Transcriptomic Alterations with Corresponding Protein Expression Levels

The expression levels of hub genes in different subtypes were compared to normal samples. Results demonstrated a significant upregulation of *PLK1, KIF4A, CDCA5, CHEK1, UBE2C, CDT1, SKA3, AURKB,* and *PTTG1* in TNBC samples compared to normal ones (Fig. 5A, FDR < 0.01). *DNAAF1, CFAP70, RSPH1,*

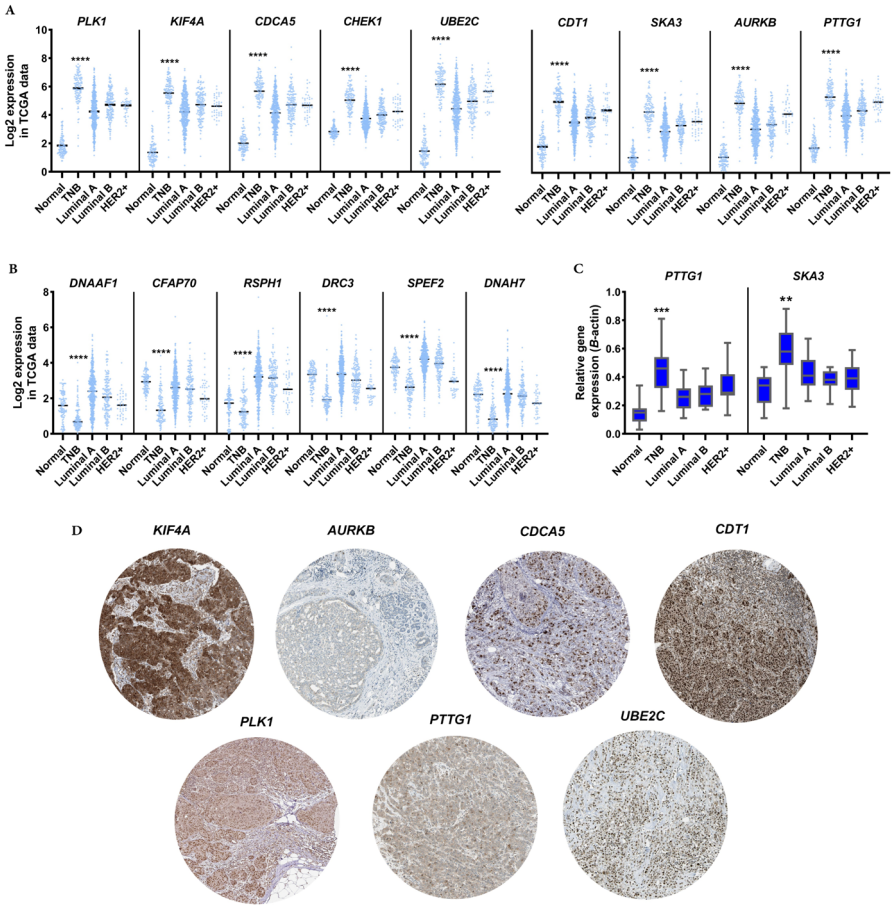

Fig. 5 A significant increase in the expression of *SKA3* and *PTTG1* in TNBC compared to other subtypes of BC. (**A**, **B**) Box plots illustrating the expression changes of hub genes identified for TNBC are presented. **C** The expression levels of *SKA3* and *PTTG1* in TNBC samples compared to healthy, luminal A, luminal B, and HER2+ samples are shown using the RT-qPCR method. The expression levels of *SKA3* and *PTTG1* were significantly higher in TNBC samples compared to other subgroups. **D** The protein expression status related to the candidate genes for TNBC is depicted based on immunohistochemistry data from the human protein atlas. As indicated in section D, the expression of proteins related to candidate genes was significantly increased in BC samples

DRC3, SPEF2, and *DNAH7* showed decreased expression levels compared to normal samples (Fig. 5B, FDR < 0.01). The protein levels of these candidate genes in BC samples were investigated using data from the human protein atlas database. As depicted in Fig. 5D, the protein levels of *PLK1, KIF4A, CDCA5, UBE2C, CDT1, SKA3, AURKB*, and *PTTG1* were significantly elevated in BC samples. However, due to inconclusive findings on *CHEK1* protein levels, it was excluded from further analysis. The downregulated genes *DNAAF1, CFAP70, RSPH1, DRC3, SPEF2*, and *DNAH7* were excluded from additional investigation since no related protein levels were discovered for them. To verify previous findings, we used RT-qPCR to measure the expression levels of SKA3 and PTTG1 in BC samples and their subgroups. These two genes were selected for study because they have received less attention in BC research. A significant increase in the expression levels of *SKA3* and *PTTG1* was observed in TNBC samples compared to normal, luminal A, HER2+, and luminal B subtypes (Fig. 5C, $P < 0.01$). These findings indicate that *PLK1, KIF4A, CDCA5, UBE2C, CDT1, SKA3*, *AURKB*, and *PTTG1* have significant therapeutic and diagnostic potential within the TNBC subgroup. We evaluated the specificity and sensitivity of the expression levels of these 8 genes in the TNBC subgroup in comparison to other subgroups (including HER2+, luminal A, and luminal B) using ROC analysis. The results for all 8 genes indicated good sensitivity and specificity in distinguishing TNBC samples from other subgroups (Fig. 6, AUC > 0.8 and $P < 0.01$). Furthermore, *AURKB* showed higher sensitivity and specificity among the candidate genes.

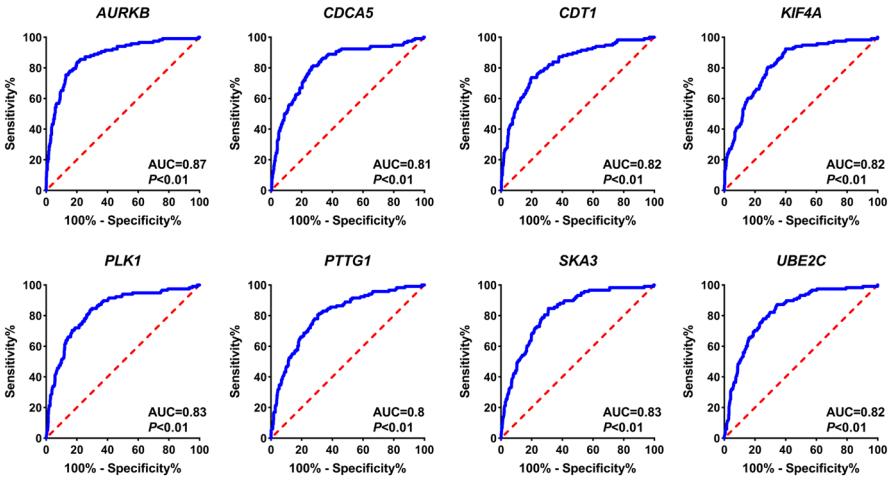

Fig. 6 The high sensitivity and specificity of candidate genes in distinguishing TNBC samples from other subtypes. The ROC curves for candidate genes in the TNBC subgroup versus other subgroups are shown. All candidate genes demonstrated acceptable AUC values

Association of Candidate Gene Expression with Drug Sensitivity to GSK 461364 and IKK 16

We investigated the association of expression levels of candidate genes, including *PLK1, KIF4A, CDCA5, UBE2C, CDT1, SKA3, AURKB*, and *PTTG1*, with drug resistance and sensitivity via PharmacoDB data. Results indicated that none of the candidate genes were associated with resistance to common chemotherapeutic drugs for BC. Our findings showed that high expression levels of genes like *PLK1, CDCA5, UBE2C, CDT1,* and *AURKB* were linked to sensitivity to GSK 461364 (Fig. 7A, $R > 0.4$ and FDR < 0.01). High expression levels of *PLK1, UBE2C, AURKB*, and *PTTG1* showed a significant correlation with sensitivity to IKK 16 (Fig. 7A, $R > 0.4$ and FDR < 0.01). Furthermore, our candidate genes exhibited significant associations with drug sensitivity to Ispinesib, Oxamflatin, Olomoucine II, Ixabepilone, Doxorubicin, and PD 173074 (Fig. 7A, FDR < 0.01). Analysis of GEO data indicated that Bortezomib can significantly decrease the expression levels of *CDCA5, PLK1,* and *PTTG1* (Fig. 7B, FDR < 0.05). These findings indicate that the mentioned drugs have promising potential for targeted treatment in the TNBC subgroup.

Discussion

The TNBC subtype has distinct molecular characteristics compared to other breast cancer subtypes, and most cancer cells in this category do not respond to common BC treatments (Collignon et al. 2016). In this study, we utilized in silico data and RT-qPCR methodology to identify genes within the TNBC subgroup with promising therapeutic potential. Additionally, we assessed drug sensitivities associated with TNBC-linked candidate genes.

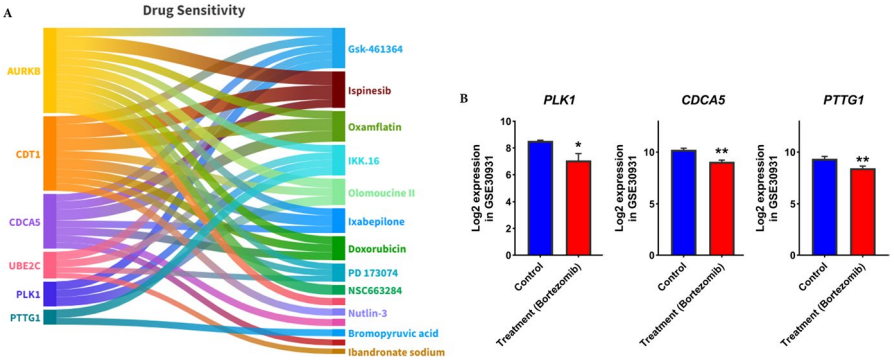

Fig. 7 The correlation between increased expression of candidate genes and sensitivity to GSK 461364 and IKK 16 drugs. **A** The relationship between increased expression of candidate genes and sensitivity to different drugs is presented based on PharmacoDB data. The wider lines suggest a reduced false discovery rate (FDR). **B** Decreased expression of certain candidate genes, such as *PLK1*, *CDCA5*, and *PTTG1*, is demonstrated in response to bortezomib treatment in breast cancer cell lines

Upregulated genes in the TNBC subgroup, compared to luminal A, luminal B, and HER2+ subgroups, are involved in inflammation, hypoxia, and key cellular proliferation pathways such as E2F targets, the mitotic spindle, and the G2-M checkpoint. The E2F family, a group of transcription factors, controls the expression of genes related to cell proliferation. The expression of E2F target genes and E2F1 is significantly elevated in TNBC compared to other subgroups (Verlinden et al. 2007). A comprehensive study has demonstrated that the expression levels of genes related to inflammation, angiogenesis, and cell proliferation, such as mitotic spindle and G2-M checkpoint, are elevated in the TNBC subgroup compared to others (Ossovskaya et al. 2011). A recent report has highlighted the prominent role of hypoxia-related genes like *HIF1A* in the development and invasiveness of TNBC compared to other subtypes and has suggested hypoxia pathways as potential therapeutic targets for TNBC (Liu et al. 2022). There are also studies indicating that genes related to cell proliferation pathways, inflammation, and hypoxia are significantly upregulated in TNBC (Liu et al. 2022; Mustafa et al. 2024; Ossovskaya et al. 2011). These findings indicate that cell proliferation, inflammation, and hypoxia pathways play significant roles in the TNBC subgroup. Genes related to these pathways could be suitable therapeutic and diagnostic targets for this specific BC subclass.

Our investigations revealed that eight upregulated genes, including *PLK1, KIF4A, CDCA5, UBE2C, CDT1, SKA3, AURKB,* and *PTTG1*, have the potential to serve as hub genes in TNBC. Additionally, survival analysis results indicated a poor prognosis associated with the mentioned genes in patients. Data analysis from the human protein atlas revealed a significant increase in the expression levels of proteins related to these genes in BC samples. RT-qPCR results demonstrated significantly higher expression levels of *SKA3* and *PTTG1* in TNBC samples compared to healthy, luminal A, luminal B, and HER2+ samples. Examining the sensitivity and specificity of *PLK1, KIF4A, CDCA5, UBE2C, CDT1, SKA3, AURKB*, and *PTTG1* gene expression levels demonstrated their potential to effectively differentiate TNBC samples from other subtypes. *PLK1* is essential for mitosis. Its dysfunction can promote cancer progression, and its overexpression is linked to poor prognoses in various cancers (Liu et al. 2017). It has been found that the expression level of *PLK1* is increased in the TNBC subgroup compared to other BC subgroups, and inhibition of this gene has the potential to regulate cell cycle and malignancy-related pathways (Ueda et al. 2019). Multiple studies have shown that *KIF4A* is crucial for spindle organization, chromosome alignment and condensation, and cytokinesis during proliferation (Hu et al. 2011; Rath & Kozielski 2012). In addition, it has been reported that the expression level of *KIF4A* is elevated in TNBC, and its expression levels may correlate with the migration and malignancy of TNBC cells (Tang et al. 2019). *CDCA5* is a key regulatory factor for sister-chromatid separation and cohesion (Chen et al. 2020). TNBC has an enhanced expression level of *CDCA5*, and cellular migration and division may be modulated by downregulating its expression (Li et al. 2022). Further evidence suggests that *UBE2C* expression is higher in the TNBC subgroup than in other subgroups, associated with a worse prognosis (Kariri et al. 2022). *UBE2C* can contribute to cancer development by breaking down mitotic cell cyclins and advancing the cell cycle. Other studies have also indicated an increase in the expression of *CDT1* and *AURKB* in TNBC and their correlation

with malignancy (Maimaiti et al. 2022; Naorem et al. 2019). *CDT1* plays a crucial role in initiating DNA replication, which is fundamental for regulating cell proliferation in cancer (Cai et al. 2021). *AURKB* is a serine/threonine protein kinase involved in mitosis as part of the chromosomal passenger complex. It plays a crucial role in the progression of the cell cycle. Dysregulation of *AURKB* is observed in various tumors, where its increased expression is correlated with tumor cell invasion, metastasis, and resistance to therapies (Borah and Reddy 2021). In this study, we demonstrated for the first time that the expression levels of *SKA3* and *PTTG1* are significantly elevated in TNBC compared to other subgroups and normal samples. Moreover, increased expression of *SKA3* and *PTTG1* was associated with a poor prognosis for patients. *SKA3* is a component of the outer kinetochore microtubule-binding complex, crucial for accurate chromosomal segregation and cell division (Feng et al. 2024). *PTTG* is an oncogene critical for cell cycle regulation and the segregation of sister chromatids. Its abnormal expression is reported in various cancers (Parte et al. 2019). These findings suggest that *PLK1, KIF4A, CDCA5, UBE2C, CDT1, SKA3, AURKB,* and *PTTG1* are potentially valuable therapeutic targets in TNBC. All the candidate genes can play a role in the pathogenesis of TNBC through pathways related to cell proliferation.

We found a strong relationship between drug sensitivity to GSK 461364 and IKK 16 and the expression of potential genes. GSK 461364 specifically targets *PLK1* and inhibits its activity (Fernández-Sainz et al. 2023). Using GSK 461364 in TNBC effectively reduces cell invasion and growth (Giordano et al. 2019). IKK 16, an inhibitor of IκB kinase (IKK), reduces inflammatory and immune responses, cellular proliferation, and induces apoptosis by targeting NF-κB activity. Research findings indicate that IKK 16 successfully reduces TNBC cell proliferation (Zhang et al. 2022). Our investigation into GEO data revealed a significant reduction in the expression levels of *PLK1*, *CDCA5*, and *PTTG1* in BC cell lines following treatment with bortezomib. Bortezomib is a proteasome inhibitor widely used for melanoma treatment. Bortezomib inhibits the degradation of IκB, an NF-κB inhibitor, demonstrating significant anti-tumor activity against malignancies. The findings show that bortezomib can effectively inhibit cell proliferation and induce apoptosis in TNBC (Shen et al. 2015; Tseng et al. 2012). These findings suggest that drugs such as GSK 461364, IKK 16, and bortezomib have promising potential for the treatment of TNBC by targeting candidate genes. However, these results are based on in silico analysis, and further validation through in vitro and in vivo experiments is necessary, representing a primary limitation of this study. Additionally, it is suggested to investigate the simultaneous effects of GSK461364, IKK16, and Bortezomib on TNBC cells.

Conclusion

The treatment of TNBC has been challenging due to the lack of expression of ER, PR, and HER2 receptors. Our investigations revealed that genes such as *PLK1, KIF4A, CDCA5, UBE2C, CDT1, SKA3, AURKB*, and *PTTG1* have promising potential as therapeutic targets in TNBC. A significant increase in the expression of the

mentioned genes was observed in the TNBC subgroup compared to other subgroups. Moreover, these genes were identified as hub genes in TNBC, with their expression linked to a poorer patient prognosis. Our results showed a strong correlation between the expression levels of the mentioned genes and their sensitivity to GSK 461364 and IKK 16 drug. In general, our results highlighted the therapeutic potential of *PLK1, KIF4A, CDCA5, UBE2C, CDT1, SKA3, AURKB*, and *PTTG1* genes in TNBC. Based on their expression profiles, we identified potential drugs like GSK 461364 and IKK 16 for the treatment of TNBC.

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Authors' Contributions The study design was performed by N.B., A.Z. and M.M. Data analysis and experiments were done by N.B. and M.N. Interpretations of data were performed by N.B., M.N., E.S.S and M.M. Bioinformatics analysis was performed by N.B., M.N and M.K.C Manuscript writing was performed by N.B. The manuscript was finally approved by M.M and A.Z.

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Availability of Supporting Data Supporting and raw data are available upon a reasonable request to the corresponding author. No datasets were generated or analysed during the current study.

Declarations

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

Ethical Approval and Consent to Participate All bioethical issues were reviewed and confirmed by the review board of Emam Khomeini hospital according to the criteria of the ministry of health and medical education of Iran. Written consent forms were acquired from all individuals participating in the study. Also, ethical considerations were approved by the committee related to access number IR.IAU.SHK.REC.1401.091.

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