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Identification of TIG1 associated molecular targets for breast cancer using bioinformatic approach

Meme Kanserinde Biyoinformatik Yaklaşım Kullanılarak TIG1 ile İlişkili Moleküler Hedeflerin Belirlenmesi

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ABSTRACT

Tazarotene-induced gene 1 (TIG1) is involved in modulating the α-tubulin modification and effectively inhibiting tumor growth. In this bioinformatics study, we aim to propose novel therapeutic targets in breast cancer by utilizing differentially expressed genes (DEGs) of TIG1 in inflammatory breast cancer (IBC) and examining their correlation with the molecular and immune subtypes. Using the GEO2R tool, we analyzed DEGs in the GSE30543 dataset, specifically comparing suppressed TIG1 groups with control samples from SUM149 cells. Functional annotation analysis of DEGs were explored using SRplot with data from STRING (|log2(FC)| >2 and p<0,05). Cytoscape software was used to construct intersected protein-protein interaction (PPI) network and define central genes. Subsequently, the molecular and immune subtype analysis were performed in TISIDB utilizing the identified hub genes. A total of 19 upregulated DEGs and 3 downregulated DEGs were identified in IBC and utilized to construct the STRING PPI network. GO analysis revealed that the biological functions of the identified DEGs primarily centered around the regulation of cell adhesion and migration. KEGG pathway analysis demonstrated their significant involvement in regulation of cell adhesion-related signaling pathways. Hub genes were identified as STAT3, PXDNL, FN1, CTNNB1, CD44, TNF, TP53, MMP9, SRC and INS. TISIDB analysis revealed significant correlations between all hub gene expressions and both the molecular subtypes (except for TP53) and immune subtypes of breast cancer (p<0,05). This study identified TIG1-associated hub genes as potential prognostic biomarkers for breast cancer, suggesting their utility in targeted therapies.

Keywords: Biomarker, Breast cancer, GEO, TIG1

ÖZ

Tazaroten-indüklü gen 1 (TIG1), α-tubulin tirosinasyon döngüsünü modüle etmek ve tümör büyümesini etkili bir şekilde inhibe etmekle ilişkilidir. Bu biyoinformatik çalışmada, TIG1'in inflamatuar meme kanserindeki (IBC) farklı olarak ifade edilen genlerinin (DEG'ler) moleküler ve immün alt tiplerle olan korelasyonlarını inceleyerek meme kanserinde yeni terapötik hedefler önermeyi amaçlamaktayız. GEO2R aracını kullanarak, GSE30543 veri setindeki DEG'ler analiz edildi ve özellikle baskılanmış TIG1 grupları SUM149 hücrelerinden kontrol örnekleriyle karşılaştırıldı. DEG'lerin fonksiyonel annotasyon analizi, SRplot aracılığıyla STRING verileri (|log2(FC)| >2 ve p<0,05) kullanılarak gerçekleştirildi. Cytoscape yazılımı, kesişen protein-protein etkileşim (PPI) ağını oluşturmak ve merkezi genleri belirlemek için kullanıldı. Ardından, moleküler ve immün alt tip analizleri, belirlenen merkezi genleri kullanılarak TISIDB'de gerçekleştirildi. IBC'de toplamda 19 yukarı regüle DEG ve 3 aşağı regüle DEG belirlendi ve bunlar yardımıyla STRING PPI ağı oluşturuldu. GO analizi, belirlenen DEG'lerin biyolojik işlevlerinin başlıca olarak hücre adezyonu ve göçünün düzenlenmesine odaklandığını ortaya koydu. KEGG yolak analizi ise DEG'lerin hücre adezyonu ile ilişkili sinyal yollarının düzenlenmesinde önemli bir rol oynadığını gösterdi. Merkezi genler STAT3, PXDNL, FN1, CTNNB1, CD44, TNF, TP53, MMP9, SRC ve INS olarak belirlendi. TISIDB analizi, tüm merkezi gen ekspresyonları ile meme kanserinin hem moleküler alt tipleri (TP53 hariç) hem de immün alt tipleri arasında anlamlı korelasyonlar olduğunu ortaya koydu (p<0,05). Bu çalışma ile TIG1 ile ilişkili DEG'lerden elde edilen merkezi genlerden yola çıkarak meme kanseri için hedefe yönelik terapötik yaklaşımlarda kullanılabilecek potansiyel prognostik biyobelirteçlerin belirlenmesini sağlandı.

Anahtar kelimeler: Biyobelirteç, GEO, Meme kanseri, TIG1

Biyoinformatik çalışma olduğu için Etik izin gerekli değildir.

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INTRODUCTION

Breast cancer stands as the primary contributor to cancer-related mortality in women on a global scale.[1] Inflammatory Breast Cancer (IBC), known for its accelerated growth and distinctive clinical and biological features, represents the highest aggressive breast cancer. IBC is marked by elevated risks of metastasis and recurrence, coupled with a diminished survival than non-IBC forms.[2]Within IBC, the triple-negative subtype (TN-IBC) is notably recognized for its particularly aggressive nature. TNBC is observed in approximately 10% to 20% of non-IBC patients, while the prevalence is higher in IBC, ranging from 20% to 40% of patients with TN-IBC.[2,3]

Numerous molecular alterations have been identified as contributors to the aggressive nature of IBC, such as overexpression of translation initiation factor eIF4GI, Rho C GTPase and E-cadherin and the loss of WISP3.[3] Besides these, tazarotene-induced gene 1 (TIG1), also referred as retinoic acid receptor responder 1, demonstrates high expression in both TNBC and IBC.[4] TIG1 is a member of the latexin family, which comprises putative cytoplasmic carboxypeptidase inhibitors. Studies have shown that TIG1 plays a role in the modulation of the α-tubulin tyrosination cycle through its interaction with the ATP-GTP binding protein-like 2 (AGBL2) protein.[5] It exhibited inhibitory impacts on the proliferation and invasion of diverse cancer cell types. The lost/silence or downregulation of TIG1 expression in various carcinomas including head and neck cancer, endometrial cancer, prostate cancer and colorectal cancer was attributed to the CpG hypermethylation in the promoter site.[6,7] The ectopic expression of TIG1 has been displayed to inhibit cancer development.[8,9] Thus, TIG1 could potentially have a significant function in retinoic acid-induced cellular differentiation and the inhibition of tumor growth.[6] Moreover, it has been identified as candidate gene with tumor-suppressive properties in both endometrial and prostate cancer.[8,10]

Understanding the molecules and mechanisms involved in carcinogenesis has the potential to improve cancer prediction and prognosis, and the enhancement of targeted prevention and treatment strategies. Innovative molecular analyses, including high-throughput techniques like cDNA microarray and proteomics, have significantly advanced the detection and characterization of differentially expressed genes (DEGs) and proteins in normal and malignant cells.[11] Due to the molecular and clinical heterogeneity observed in breast cancer, DEG analyses hold significance for this particular type of cancer. While numerous biomarkers have enhanced treatment effectiveness, there remains a pressing need to identify novel therapeutic targets highly responsive to breast cancer.[12,13] The dataset GSE30543, available in the Gene Expression Omnibus (GEO) database, presents expression profiles of SUM149 cells (TN-IBC cells) transfected with siRNA targeting TIG1 and SUM149 cells transfected with control siRNA through array analysis. In the research that produced this dataset, it was concluded that TIG1 enhances the malignant characteristics of IBC by inducing Axl functionality.[7]

Since the role of TIG1 in breast cancer remains unclear, and the mechanisms underlying its tumor-suppressive effects remain largely unexplored, we performed the identification of DEGs between suppressed TIG1 groups and control samples in GSE30543 dataset. Later on, in silico analysis of DEGs were conducted to uncover gene enrichment in IBC. To shed light on the signaling network it might create for other subtypes of breast cancer, the IBC DEGs were intersected with the breast cancer network, and the hub genes in this intersected PPI were identified. Subsequently, correlation analysis was conducted to examine the expression of these hub genes in molecular and immune subtypes of breast cancer. Thus, by commencing with IBC, the bioinformatic approaches will be employed to elucidate the signaling network and immune effects

associated with the TIG1 gene, the role of which remains incompletely understood in breast cancer.

MATERIALS AND METHODS

Data collection and processing

The Gene Expression Omnibus (GEO), a genomics database accessible through the National Center for Biotechnology Information (NCBI), was extensively searched to retrieve all datasets related to studies on breast cancer. The GSE30543 dataset provided cDNA microarray data of SUM149 cells with control siRNA (siControl) and SUM149 cells transfected with siRNA that targets TIG1 gene (siTIG1 group) through Affymetrix Human Genome U133 Plus 2.0 Array. The GEO2R module, utilizing the limma algorithm in the R programming, was employed to identify DEGs between the control and siTIG1 groups.

The samples in GSE30543 dataset were normalized and the cutoff criteria for identifying statistically significant DEGs were set as a $p<0,05$ and $|logFC|>2$. Consequently, hierarchical heatmap analysis of the expression levels of the identified DEGs was conducted using SRplot to reveal variations in expression patterns among different samples and groups.[14]

Construction of protein-protein Interactions

The protein-protein interaction (PPI) network of DEGs was generated utilizing Search Tool for the Retrieval of Interacting Genes/Proteins (STRING). Evidence based network edges were created with medium confidence level (0,400). Moreover, PPI network of DEGs and breast cancer were constructed in Cytoscape v3.10.1 using STRING database. A maximum of 100 additional interactors and a confidence threshold of 0,4 were chosen for the analysis. Subsequently, the constructed PPI networks were intersected to obtain common interactors.

The Maximal Clique Centrality (MCC) algorithm within the cytoHubba plugin was employed to identify the top 10 hub genes from the combined PPI network.

Analyzing the functional annotation of DEGs

The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were analyzed using the SRplot.[14] These analyses were conducted using top 10 terms based on FDR values in the STRING. GO annotation was conducted to assess biological processes (BP), cellular components (CC), and molecular functions (MF) subgroups associated with the DEGs. KEGG was used to identify biological pathways for DEGs. $p<0,05$ were considered statistically significant.

Correlation of the expression of hub genes with molecular and immune subtypes of breast cancer

Tumor-immune system interactions database (TISIDB) is a web-based platform designed to investigate the interaction between cancer cells and the immunity.[15] We utilized this resource to examine the relationship between the expression of core genes and molecular and immune subtypes of breast cancer. TISIDB comprises five molecular subtypes; basal, Her2, luminal A & B, normal. The immune subtypes within TISIDB are classified as follows: C1, which corresponds to wound healing; C2, characterized by IFN-gamma dominance; C3, representing an inflammatory subtype; C4, indicating lymphocyte depletion; C5, denoting an immunologically quiet state; and C6, exhibiting dominance of TGF-β. The log2-transformed counts per million (log2CPM) expression values in the context of RNA-Seq data analysis were obtained for different subtypes. $p<0,05$ is evaluated as statistically significant difference.

RESULTS AND DISCUSSION

Identification of DEGs

The results of GSE30543 dataset analysis were represented in Figure 1. The boxplot demonstrated that the interquartile ranges and medians of gene expression values in the two groups were close to each other. (Figure 1a). Among the 33 differentially expressed genes (DEGs) identified, FBXO32, GBP1, and EGR1 exhibited repetitive outcomes, and certain data points lacked informative value. Consequently, 3 genes were defined as downregulated, while 19 genes were found to be upregulated in siTIG1 compared to the control group (Figure 1b, Table 1). Hierarchical cluster analysis clearly separated the siTIG1 and control group (Figure 1c). Although there were variations observed among the biological samples, the samples predominantly clustered into two main groups.

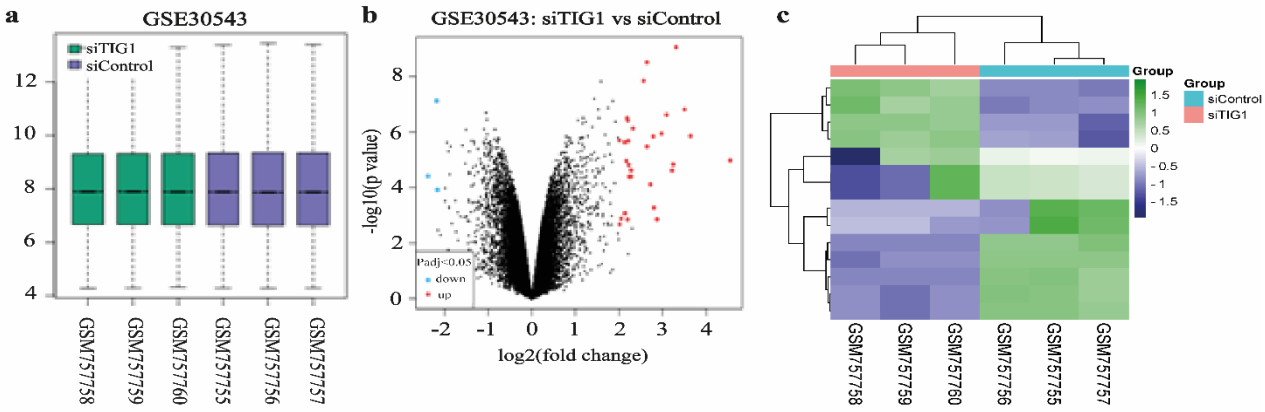

Figure 1. Identification of DEGs. a. The boxplot represents the distribution of gene expression levels in each sample of GSE30543. b. Volcano plot of the expression level of DEGs in siTIG1 and siControl groups from GSE30543 dataset. Blue dots indicate genes with a statistically significant low expression, while red dots represent genes with a statistically significant high expression. c. The heatmap reveals the expression patterns of DEGs. The x-axis corresponds to individual samples and the y-axis represents the selected gene. Overexpression is depicted by green blocks and lower expression levels are represented by blue blocks.

Table 1. The downregulated and upregulated DEGs in siTIG1 and siControl group.

Downregulated genes	Gene ID	LogFC	Adjusted p-value
NME7	29922	-2,18	0,0006043
SHISA2	729993	-2,382	0,0065637
PHLDA1	22822	-2,162	0,0110883
Upregulated genes			
FBXO32	114907	3,501	0,0008487
GBP1	2633	3,242	0,0043962
SERPINB4	6318	3,216	0,0053116
NPY1R	4886	2,975	0,0022475
FN1	2335	2,872	0,036831
MMP7	4316	2,801	0,0219174
EGR1	1958	2,786	0,0022475
SERPINB3	6317	2,721	0,0088453
DCLK1	9201	2,643	0,0025582
OLFML3	56944	2,319	0,0017605
SERPINB4/// SERPINB3	6318/// 6317	2,275	0,0053116
SULT1E1	6783	2,223	0,0011953
CFI	3426	2,186	0,0011606
DCN	1634	2,168	0,003829
STEAP4	79689	2,137	0,0278274
LINC00284	121838	2,131	0,0022475
CD24	100133941	2,052	0,0358452
CENPW	387103	2,015	0,0456422
SERPINA3	12	2,007	0,0022475

PPI network and functional annotations of DEGs

The network constructed using 22 DEGs based on STRING database included 50 nodes and 235 edges (Figure 2a). The GO results for biological process (BP) revealed that DEGs associated with TIG1 were mainly involved in cell and substrate adhesion, cell-matrix adhesion and cell adhesion by integrin, positive regulation of cell migration, cell

migration. The GO cellular component (CC) annotations showed that TIG1 were notably associated with extracellular space, cell adhesion-related proteins, integrin complex, vesicle and cell surface. Regarding GO molecular function (MF), DEGs-correlated with TIG1 were implicated with various functions such as binding to integrins, fibronectin, extracellular matrix

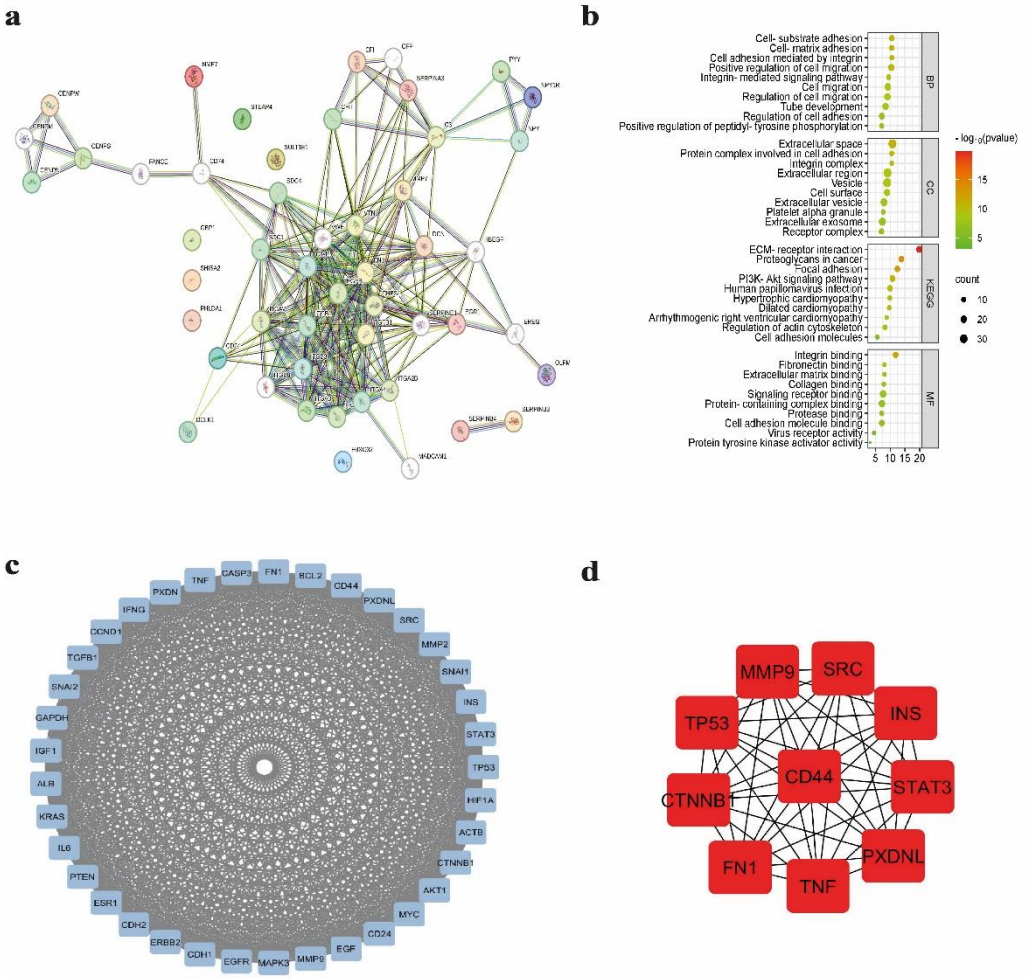

Figure 2. PPI network and functional enrichment analysis of DEGs. a. The DEGs network derived from the STRING database. b. Functional enrichment analysis. Red highlights the most significant processes, while green indicates less significance based on -log10(p-value). The size of the dots on the graph correlates with the number of genes involved, with larger dots indicating a higher gene count. BP: Biological process, CC: Cellular component, MF: Molecular function c. Intersected pathway constructed by merging breast cancer targets and DEGs networks. d. The network generated by MCC algorithm for the 10 hub genes of intersected pathway.

(ECM), collagen, cell adhesion molecules. The results obtained from the KEGG pathway analysis indicated that TIG1 was involved in ECM-receptor interaction, regulation of the cell cytoskeleton, cell adhesion-related signaling pathways, as well as in the PI3K-Akt and cardiomyopathy signaling pathways (Figure 2b). The PPI network, generated by intersecting breast cancer targets and DEGs networks, comprised 37 nodes and 658 edges (Figure 2c). The 10 core genes in the intersected PPI were as follows: STAT3, PXDNL, FN1, CTNNB1, CD44, TNF, TP53, MMP9, SRC, INS (Figure 2d).

Association between the expression of hub genes and molecular and immune subtypes of breast cancer

According to an analysis on the TISIDB, all hub gene expressions were significantly associated with the molecular subtypes of breast cancer ($p<0,05$), except for TP53 ($p=0,425$). For six immune subtype (C1-C6) correlations in breast cancer, the significant

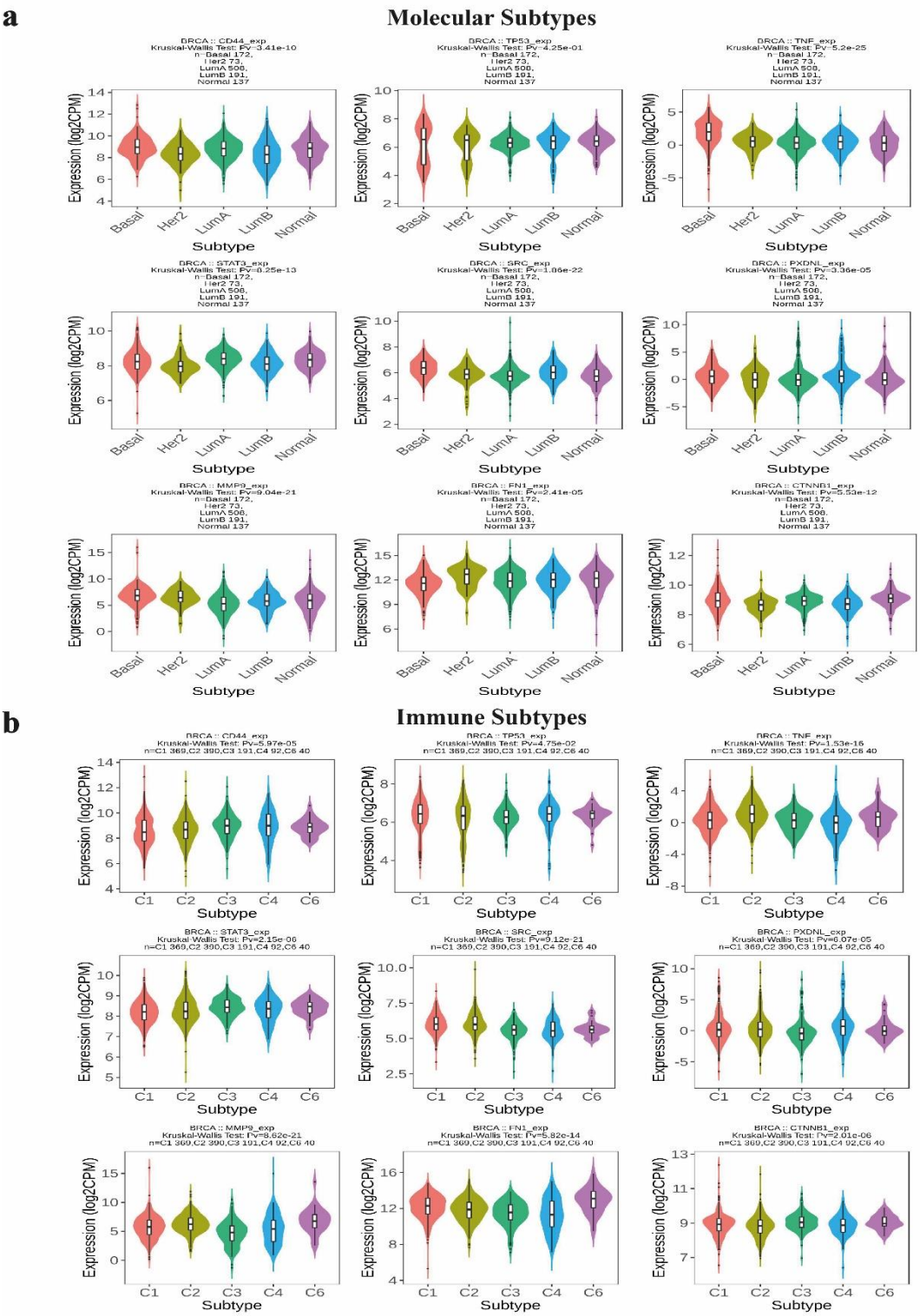

Figure 3. Violin plots represent the correlation of hub gene expression with molecular (a) and immune (b) subtypes of breast cancer. p<0,05 implies statistically significant difference.

relationship was obtained for all of the core genes (p<0,05) (Figure 3). Table 2 includes the expression profiles of individual hub genes within the immune subtypes. TISIDB database does not have available data for the specific correlation between INS gene and both subtypes in breast cancer. Therefore, the association was obtained for nine hub genes.

Table 2. The expression status of hub genes in five immune subtypes for breast cancer.

Hub genes	Full Name	Highly expressed	Lowly expressed
CD44	CD44	C4	C1
TP53	Tumor protein P53	C1	C3
TNF	Tumor necrosis factor	C2	C4
STAT3	Signal transducer and activator of transcription 3	C6	C1
SRC	SRC proto-oncogene	C1	C4
PXDNL	Peroxidasin like	C4	C3
MMP9	Matrix metallopeptidase 9	C6	C3
FN1	Fibronectin 1	C6	C4
CTNNB1	Catenin (cadherin-associated protein) beta 1	C3&C6	C2

The discovery of molecular biomarkers in breast cancer is vital for improving treatment strategies. While existing biomarkers have enhanced breast cancer treatment, there's a pressing need for new responsive prognostic biomarkers and therapeutic targets due to the heterogeneous nature of breast cancer.[12,16,17] Assessing DEGs that offer discriminative candidate genes is a critical step in the discovery of novel biomarkers.[18] The primary objective of the present analysis is to merge the PPI network of DEGs based on TIG1 from the GSE30543 dataset specific to IBC with the PPI network covering all subtypes of breast cancer to reveal hub target genes. Furthermore, the study also aims to examine correlations between these identified key targets within the merged PPI network and immune/molecular subtypes, utilizing bioinformatic approaches.

The data from the resulting 33 DEGs in GSE30543 were comparable between the two groups, and the variability of gene expression values was consistent across both groups. Moreover, the heatmap revealed well-separated groups forming distinct clusters, suggesting the distinct expression patterns among the groups. Therefore, identification of biological functions and pathways correlated with the DEGs were critical to understand the functional implications of the observed patterns. Although some DEGs such as GBP1, SHISA2, and PHLDA1 were not included in the PPI analysis, most DEG members were part of the signaling network based on experimentally determined and curated databases. When a functional enrichment analysis was performed on this PPI network's data, the GO results notably showed that TIG1 associated DEGs plays a significant role in cell adhesion and cell migration. These results align with the findings of the team that generated the GEO30543 dataset, particularly their discovery that silencing endogenous TIG1 reduces aggressiveness of IBC cells *in vitro*. They observed that depletion of TIG1 in IBC cells resulted in decreased Axl expression, downregulation of MMP-9 and NF-κB inactivation. Additionally, inhibiting Axl pathway resulted in decreased cell growth, movement, and invasion capabilities of IBC cells, consistent with various other studies.[7,19,20] On the other hand, our KEGG pathway results are in concordance with both the literature and GO analysis, indicating that TIG1-associated DEGs primarily participate in the regulation of cell cytoskeleton and cell adhesion signaling pathways. The KEGG analysis also revealed the effectiveness of DEGs in the phosphatidylinositol 3 kinase and protein kinase B (PI3K-AKT) pathway, which modulates various cellular processes, including survival, glucose metabolism migration, proliferation, invasion, apoptosis, and DNA repair.[21] PIK3CA emerges as a commonly mutated gene in different human tumors, encoding the p110α catalytic subunit of the PI3K pathway across various neoplasms. Amplification of this gene has been identified in cancers such as breast cervical, head and neck, lung and gastric cancer. The highest incidence of PIK3CA mutations has been observed in colon, breast, endometrium, and prostate cancers. Ongoing clinical studies are currently assessing inhibitors targeting the PI3K/AKT/mTOR axis for potential therapeutic interventions.[21,22] The demonstration in our study that TIG-1-associated DEGs may exert an influence on this pathway, which holds great significance for carcinogenesis, could offer a novel perspective for the treatment and management of breast cancers.

The intersected PPI network was constructed to identify common interactors of TIG1-associated DEGs and breast cancer. This revealed which proteins are shared in the general breast cancer pathway encompassing all subtypes of proteins associated with TIG1 in IBC. Among the central proteins identified, MMP9, previously shown to be involved in the TIG1 axis in IBC, is suggested to be a central regulator for breast cancer as a whole.[7] MMP9 is crucial for breaking down type IV collagen, a key constituent of the basement membrane. It actively participates in tumor invasion, modulating the tumor microenvironment (TME) and stimulating tumor-induced angiogenesis. Prior research has linked elevated MMP9 expression with the onset of metastases in breast cancer patients, leading to an unfavorable prognosis.[23] The MMP-9 expression level has shown a connection with the extent of activated STAT3 in breast cancer in human subjects, and activated by uPA, a vital enzyme that converts plasminogen to plasmin, leading to ECM degradation. Elevated uPA levels are associated with shorter disease-free periods in breast cancer patients. Recent studies correlate uPA overexpression to highly invasive basal-like breast cancer through a CD44-associated mechanism. Protein microarray analysis also indicates a notable correlation between uPA and STAT3 expression in primary breast cancer tissue.[24] Thus, the MMP9, CD44, and STAT3 hub genes identified in the PPI network have been shown to have crucial roles in breast cancer. Furthermore, in contrast to most cancer types, ER+ breast cancer cells frequently maintain wild-type p53. Given the crucial role of tumor suppressor p53 loss of function in cancer development, it is hypothesized that cancer cells expressing wild-type p53 may possess mechanisms to suppress its function. SRC has been indicated to promote cell proliferation in ER+ breast cancer by inhibiting p53 function.[25] The five remaining core interactors—PXDNL, FN1, CTNNB1, TNF, and INS—within the PPI network were also identified as significant contributors to the breast carcinogenesis.[26–28] Taken together, in our pathway analysis, emphasis has been placed on a set of target proteins with effects that have been illuminated or are still being elucidated in breast cancer.

Anticipating the prognosis is essential for cancer care and poses a challenge for many cancers due to the limitations of clinicopathologic factors. Gene expression patterns that can predict the course of a disease play a pivotal role in enhancing patient treatment by categorizing tumors into distinct groups, thereby offering insights for personalized treatment decisions.[29] On the basis of this information, we analyzed the correlation between the expressions of 10 hub genes and breast cancer molecular subtypes. Upon analyzing the significant findings, it was identified that the expression of PXDNL, CD44, SRC, MMP9, and TNF hub genes was elevated in the basal subtype. These hub genes may be associated with specific pathways or processes that are more active or dysregulated in basal breast cancer, potentially contributing to its unique characteristics. While the expressions of STAT3 and FN1 were highest in Luminal A and HER2 subtypes, respectively, the expression of CTNNB1 was found to be highest in normal breast tissue. The lower expression of CTNNB1 in breast cancer subtypes might indicate a loss of regulatory control, contributing to the development and progression of cancer.

Immunotherapy has introduced a novel approach to cancer treatment, but its effectiveness varies among cancer types and individuals. Understanding the immune system response within the TME is crucial for informing immunotherapy drug development and clinical strategies. In addition to gene expression variations among BRCA molecular subtypes, there are substantial variations in the cellular constitutions within the TME, particularly immune cells. These differences have a direct impact on both patient prognosis and the response to therapy.[30] The immune subtypes (C1-C6) characterize distinct immune features and mechanisms, providing a classification of breast cancer cases based on the nature of the immune response.[31] In the current research, higher expression of the core genes was

predominantly correlated with the C6 immune subtype. This implies that these particular genes play a significant role or are closely associated with the immune features and mechanisms of the C6 subtype which is characterized by dominance in TGF-β signaling. It exhibited the most prominent TGF-β signature along with a substantial lymphocytic presence, featuring a balanced distribution of both type I/II T cells.[30] On the other hand, lower expressions of hub genes were significantly associated with C3 and C4 subtypes, which are inflammatory and lymphocyte depleted subtypes, respectively.[30] From a general perspective, the significant association of all hub genes derived from the intersected PPI network of TIG1-associated DEGs with immune subtypes implies a comprehensive relationship between these genes and distinct immunological features within the TME. This correlation also suggests that the expression signatures of the central genes may be indicative of specific immune responses or characteristics in breast cancer, providing valuable information for understanding the interplay between the tumor and the immune system.

TIG1 associated DEGs demonstrated significant role in regulation of cell adhesion and cell migration through various important pathways such as PI3K/AKT signaling. Hub genes were identified to transform the data from IBC to all breast cancer subtypes and subsequently new set of target proteins for breast cancer were demonstrated as STAT3, PXDNL, FN1, CTNNB1, CD44, TNF, TP53, MMP9, SRC and INS. Their higher expression was found predominantly in basal subtype and their higher expression was predominantly correlated with C6 immune subtype.

CONCLUSION AND RECOMMENDATIONS

In conclusion, starting with IBC, we elucidated the signaling network and unique immune characteristics associated with the TIG1 gene across all breast cancer subtypes. TIG1-associated DEGs was revealed to play a significant role in regulating cell adhesion and migration through crucial pathways such as PI3K/AKT signaling. The identification of the hub genes facilitated the translation of data from IBC to encompass all breast cancer subtypes, leading to the identification of a new set of target proteins for breast cancer. Furthermore, understanding the gene expression patterns of hub genes within each molecular and immune subtype provides valuable prognostic information and detailed comprehension of the immune environment in breast cancer, respectively. Overall, these bioinformatic analyses lay the groundwork to support future research on targeted therapies for breast cancer. The limitations of this study include the reliance on *in vitro* experiments, which may not fully replicate *in vivo* conditions. Additionally, the protein-protein interaction data used for network analysis is subject to inherent biases and potential inaccuracies, which could affect the identification of key proteins. Further validation through experimental and clinical studies is required to substantiate these findings. Upon validation through *in vitro* and *in vivo* experiments, the findings from the current study have the potential to disclose valuable biomarkers applicable for diagnostics, monitoring treatment responses, and predicting patient outcomes.

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