Uncovering Novel Gene Signatures involved in chromosomes segregation in Triple-Negative Breast Cancer using Differential Transcriptome Analysis

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Abstract

Triple-negative breast cancer (TNBC), a heterogeneous subtype of breast cancer (BC) is characterized by the absence of estrogen (ER), progesterone (PR) and HER2 receptors. TNBC and non-TNBC exhibit distinct biological behaviours, primarily due to differentially expressed genes (DEGs). This study is focusing to identify key DEGs that may have association with TNBC pathogenesis. mRNA datasets (GSE36295, GSE45255 and GSE163882) comprise of 116 TNBC and 295 non-TNBC patients and were analyzed using the "affy" R-package for expression profiling. Pathway enrichment analysis identified biological processes associated to cancer progression. Of the 998 DEGs, 169 genes were upregulated and 829 genes were downregulated with significant changes ($p < 0.001$).

Notably, several genes were identified that are linked with highly activated cancer progression pathways, among them MYBL2, KIF23, BRIP1, CENPN, KIFC1, XRCC3 and CDCA8 are associated with chromosomal segregation pathways. For the identification of regulating miRNAs, Enrichr web tool was used demonstrating that MYBL2, BRIP1 and CENPN genes are regulated by miR-4454, miR-4632 and miR-1307. Furthermore, the sensitivity and specificity of these genes were assessed utilizing receiver operating characteristic (ROC), that exhibited KIF23 and BRIP1 having highest AUC (Area Under Curve) value of 0.79 and 0.77 respectively. That suggested their strong potential as a biomarker for diagnosis of TNBC.

Keywords: Triple-negative breast cancer (TNBC), Differentially Expressed Gene (DEG), Pathway Enrichment Analysis, Oncogenic pathway and biomarkers.

Introduction

Breast cancer (BC) is the most common cancer in women and it is the second leading cause of cancer deaths worldwide[51]. Primarily, it was reported that BC originates in the ductal epithelium (ductal carcinoma), however, several studies have concluded that it can also develop in the breast lobules (lobular carcinoma)[35]. It is often observed that BC can spread much faster than other cancers, may migrate to distant organs such as lungs, liver and brain. This characteristic makes it particularly challenging to cure[45]. This is one of the primary reasons for the rising global mortality rate associated with this cancer, as the number of global deaths continues to increase yearly.

According to the WHO, the data shows that BC caused approximately 670,000 deaths globally in 2022. Remarkably, about half of all BC cases, were having no specific risk factors other than their sex and age and women from 157 out of 185 countries had been suffering with this (https://www.who.int/).

The classification of cancer helps in understanding the tumour nature and making it easier to diagnosis and predict its behaviour that helps in planning treatments [50]. Perou et al[41], used microarray technology to classify BC and they suggested that BC has five subtypes, including HER2-enriched, basal-like, luminal A, luminal B and normal breast-like subtypes. The basal-like subtype of BC has garnered significant attention due to its high prevalence and lack of effective targeted therapies. TNBC is closely associated with this subtype which is characterized by the absence of estrogen receptor (ER), progesterone receptor (PR) and HER2 expression and lack of these receptors makes treatment more challenging[50].

TNBC represent 15% of all BC[17]. Basal-like cancer, which primarily affects individuals of African American and Hispanic descent, is usually a high-grade cancer that frequently recurs and has the potential to spread to many other parts of the body. However, significant improvements have been made in the treatment of BC, such as targeted therapies for ER+ and HER2+ BC[3,49]. For the treatment of TNBC, chemotherapy is still the only systemic option. Taxane and anthracycline-based regimens are being widely and predominantly used for treatment[37].

With advanced stage of TNBC patients, their survival time decreases significantly. Previous studies have shown that TNBC is strongly associated with hereditary compared to other BC subtypes. Overall, 10% of BC cases have been associated with BRCA1 or BRCA2 mutations[22] while 35% of TNBC patients have a BRCA1 mutation and 8% have a BRCA2 mutation[33].

Previous studies emphasized that identifying genes associated with cancer, can greatly help in understanding the related pathways and this approach can have highly beneficial results in the management of cancer. Lehman et al[24,25] noticed that expression of six subtype genes was involved in TNBC, which included basal-like 1. basal-like 2. immunomodulatory, mesenchymal, mesenchymal stem-like and luminal androgen receptor [23]. Additionally, in genomic analysis of seven paired samples of metastatic TNBC, 67 known mutations were identified that were involved in signalling pathways such as cell cycle, PI3K/AKT/mTOR, RAS/MAPK and RTK/GF.

Furthermore, Principal Coordinate analysis (PCoA) identified four distinct molecular clusters based on the gene expression patterns of PI3K/AKT/mTOR pathway, with key DEGs including AKT3, GSK3B, GNA11, PI3KR1 and GNAQ. Of these, gene AKT3 was potentially involved in progression and metastasis in TNBC[28].

In a similar study, it was noticed that gene expression profiling of TNBC tumors revealed several abnormally expressed genes such as KRAS, CDK6, AKT2, IGF1R, MYC, FGFR1, CDKN2A/B, PIK3CA and CCNE1, which were associated with cell-cycle regulation and DNA repair[24]. In comparative transcriptomic analysis of TNBC and non-TNBC, it was found that there was a significant difference in the biological roles of neural functions between these subtypes. In particular, neural genes were observed to be up-regulated in TNBC, indicating greater complexity[46].

Similarly, meta-analysis of the oncoMine database (cancer microarray database) identified 206 genes that were deregulated in aggressive TNBC cases and these genes were associated with poor outcomes. Primarily, these genes were enriched for two major functions: chromosomal instability (CIN) and estrogen receptor (ER) signaling. Of these, 8 genes performed prognostic signatures in predicting outcomes of TNBC. The CIN metagene, particularly TTK, was found to have therapeutic potential and its inhibition reduced TNBC cell survival and increased the effectiveness of chemotherapy[1].

Despite extensive research, the primary causes of TNBC are still not fully understood. In this study, we have focused on identifying DEGs that may play a crucial role in TNBC progression.

Material and Methods

In this study, genome-wide gene expression datasets were utilized to investigate the differences between TNBC and non-TNBC cases. Publicly available microarray datasets were sourced from the National Centre for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO). Specifically, three messengers' ribonucleic acid (mRNA) expression datasets (GSE36295, GSE45255 and GSE163882) were analysed, comprising data from 116 TNBC patients and 295 non-TNBC patients (Table 1). All datasets were generated using the Affymetrix Human Genome U133A Array platform which provides comprehensive coverage of human gene expression.

Affy Package - Expression computation: The gene expression levels were quantified using the 'affy' Bioconductor package (https://www.bioconductor.org/packages/release/bioc/html/affy.html) in the R statistical programming language. The package performs gene expression calculations in three key steps:

1) Background correction: It eliminates background noise captured in the scanner images, ensuring that non-specific signals do not interfere with the gene expression data;
2) Normalization: It adjusts for systematic variations between chips, enabling direct comparison of expression data across different microarray chips;
3) Expression value computation: The final step involves calculating gene expression levels from probe intensities obtained from the arrays.

To identify differentially expressed genes (DEGs) between TNBC and non-TNBC patients, we employed the GEO2R web tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/). DEGs were then selected based on a $|\log2FC| \geq 1$ and adjusted p-value < 0.05. The differences between the two groups were visualized using PCA analysis. A volcano plot was generated using the ggplot2 package in R to illustrate the distribution of DEGs. The performance of the identified DEGs in distinguishing between control and tumour samples was assessed by plotting receiver operating characteristic (ROC) curves using pROC[42].

Table 1
Characteristics of datasets included in this study

GEO Dataset ID	GEO Platform Accession Number	Subjects			Types of Sample	Microarray Platform
		Patients no.	TNBC	Non-TNBC		
GSE36295	GPL6244	50	11	39	Breast cancer tissues	Affymetrix Human Gene 1.0 ST Array
GSE45255	GPL96	139	15	124	Breast tumor samples	Affymetrix Human Genome U133A Array
GSE163882	GPL18573	222	90	132	Breast tumor samples	Illumina NextSeq 500

PANTHER Database- Classification of Differentially Expressed Genes: The classification of DEGs based on their protein class was performed using the PANTHER (Protein analysis through evolutionary relationships) database (https://www.pantherdb.org/; accessed on 10 May 2024). PANTHER DB provides a comprehensive resource for the functional classification of genes and proteins. DEGs were categorized according to their protein class, allowing for a detailed understanding of the functional roles, these genes may play in the biological processes associated with the study.

ClusterProfiler- Pathway Enrichment Analysis: Pathway enrichment analysis (PEA) of DEGs was conducted using the clusterProfiler package (https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html); accessed on 10 May 2024) within the R statistical environment. The clusterProfiler facilitates the statistical analysis and visualization of functional profiles, enabling the identification of significantly enriched biological pathways associated with DEGs. Gene Ontology (GO) terms were specifically assessed to identify overrepresented biological processes, molecular functions and cellular components. The enrichment results were adjusted for multiple testing using the Benjamini-Hochberg method and pathways with an adjusted p-value < 0.05 were considered significantly enriched. This approach provided insights into the potential functional impacts and biological relevance of the DEGs in the context of the study.

The receiver operating characteristic (ROC) curve: The Receiver operating characteristics (ROC) plots were plotted to determine specificity and sensitivity for the identified key DEGs in TNBC using Stata v17.0. The statistical significance level was set at p-value < 0.05.

Identification of microRNAs (miRNAs) binding to DEGs: The Enrich web tool (https://maayanlab.cloud/Enrichr/enrich) was used to extract miRNAs targeting key identified genes, miRNAs with significant enrichment ($p < 0.05$) were extracted[7].

Results and Discussion

Identification of Differentially Expressed Genes (DEGs) in Breast Cancer: The Principal Component analysis (PCA) data revealed all DEGs of both groups and it shows significant separation between TNBC (blue) and non-TNBC (red) that are scattered left side and right end in the graph on X-axis (Figure 1). After DEGs analysis, a total of 998 genes were found to be significantly altered in between the complex phenotype of TNBC and non-TNBC groups. Of these, 169 gens were upregulated, while 829 genes were downregulated (Figure 2).

Among all DEGs, 14% of the genes were associated with metabolite conversion enzymes that include hydrolases (six genes), lyases (one gene), oxidoreductases (two genes) and transferases (seven genes) as shown in figure 3B and C. Sixteen genes (14%) were associated with gene-specific transcriptional regulators (PC00264).

Furthermore, fourteen genes (13%) were identified as protein-modifying enzymes (PC00260), nine genes (8%) with transmembrane signal receptors (PC00197) and defense/immunity proteins (PC00090), seven genes (6%) with transporters (PC00227). Six genes (5%) were associated with RNA metabolism proteins (PC00031) and eight genes (7%) with protein-binding activity modulators (PC00095) are shown in figure 3C. In addition, we also observed genes associated with other protein classes such as cytoskeletal proteins (PC00085), structural proteins (PC00211), scaffold/adaptor proteins (PC00226), DNA metabolism proteins (PC00009), cell adhesion molecules (PC00069), intercellular signaling proteins (PC00069), cellular signaling molecules (PC00069), molecule (PC00207) and calcium-binding protein (PC00060).

Previous studies have shown that several dysregulated genes were associated with BC progression, such as phosphatidylinositol transfer protein membrane-associated 1 (PITPNM1), which contains conserved phosphatidylinositol transfer domain, associated with phosphoinositide trafficking and signaling transduction in physiology. A study conducted by Liu and colleagues[32] demonstrated that PITPNM1 expression is significantly increased in BC tissue compared to normal tissue. Additionally, the overexpression of PITPNM1 was associated with poor disease progression. In the present study, we also observed the increased expression of PITPNM1 in TNBC.

Chitinase-3 like-protein-1(CHI3L1) has been shown to be highly expressed in TNBC and other solid tumors; it induces neutrophil recruitment, leading to the formation of neutrophil extracellular traps (NETs) that display restricted T cell infiltration into the tumor stroma. Ablation of CHI3L1 in BC models results into enhanced anti-tumor immune responses, delayed tumor growth, increased T cell infiltration and increased efficacy of immune checkpoint blockade (ICB) therapy[45]. Another study also observed that CHI3L1 derived from TNBC stem cells increased the expression of CTLA4 in T cells via MAF, which subsequently suppress the function of CD8+ T cells. As a result, immune escape is observed in TNBC cells[18].

Myeloid-derived suppressor cells (MDSCs) are known for their role in cancer progression, as they contribute to primary tumor growth, metastasis and suppression of the antitumor immune response. Furthermore, Kim and Chakrabarti[21] found that TNBC patients show high levels of CHI3L-secreting from PMN-MDSCs, that suggested that CHI3L plays a key role in tumor progression and metastasis by targeting IL13Rα2, resulting to altered immune response. In our study, we also noticed upregulation of CHI3L in TNBC as compared to Non-TNBC. Cytochrome P450 family 2 subfamily E member 1 (CYP2U1) plays an important role in the metabolism of various endogenous and exogenous

substances including drugs and carcinogens. Additionally, its expression has been shown in various cancers types[32]. While studying cytochrome P4502U1, Luo et al[33] found that it is closely associated with the clinicopathological features of breast carcinoma and serves as an unfavorable prognostic factor for patients and they concluded that CYP2U1 plays an important role in BC progression. Our data also demonstrated the higher expression of CYP2U1in TNBC as compared to non-TNBC.

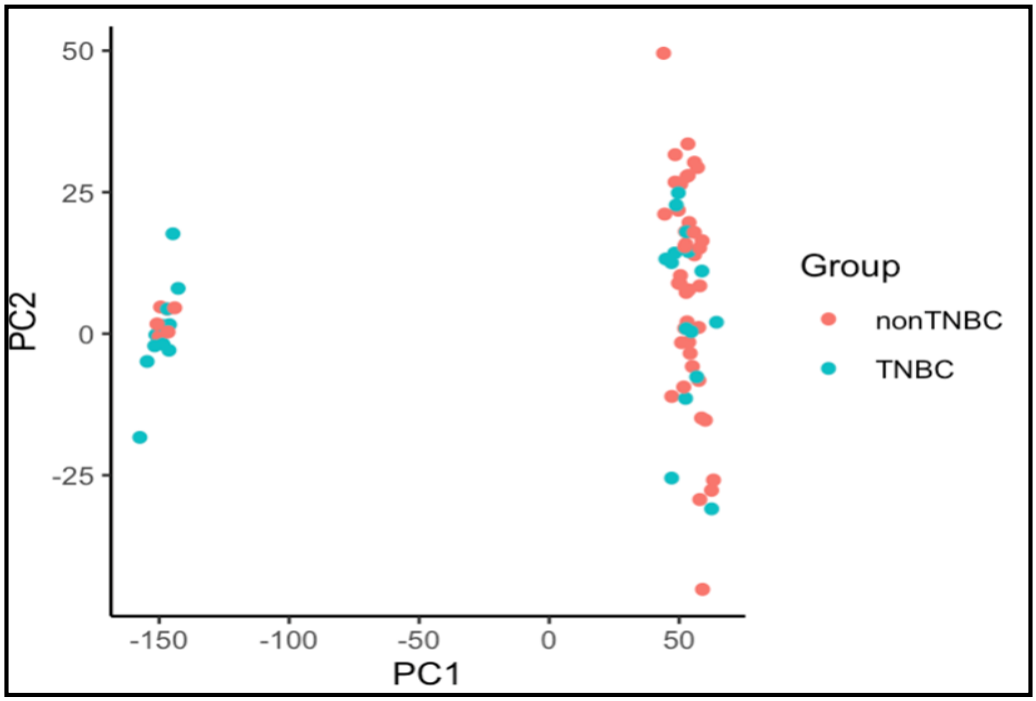

Figure 1: Principal Component (PC) analysis was conducted utilizing 998 informative genes. The significantly DEGs considered the genetic variation among TNBC and non-TNBC patients. PC1 is shown on the X and Y axis where PC1 refers to the first principal component and PC2 refers to the second principal component

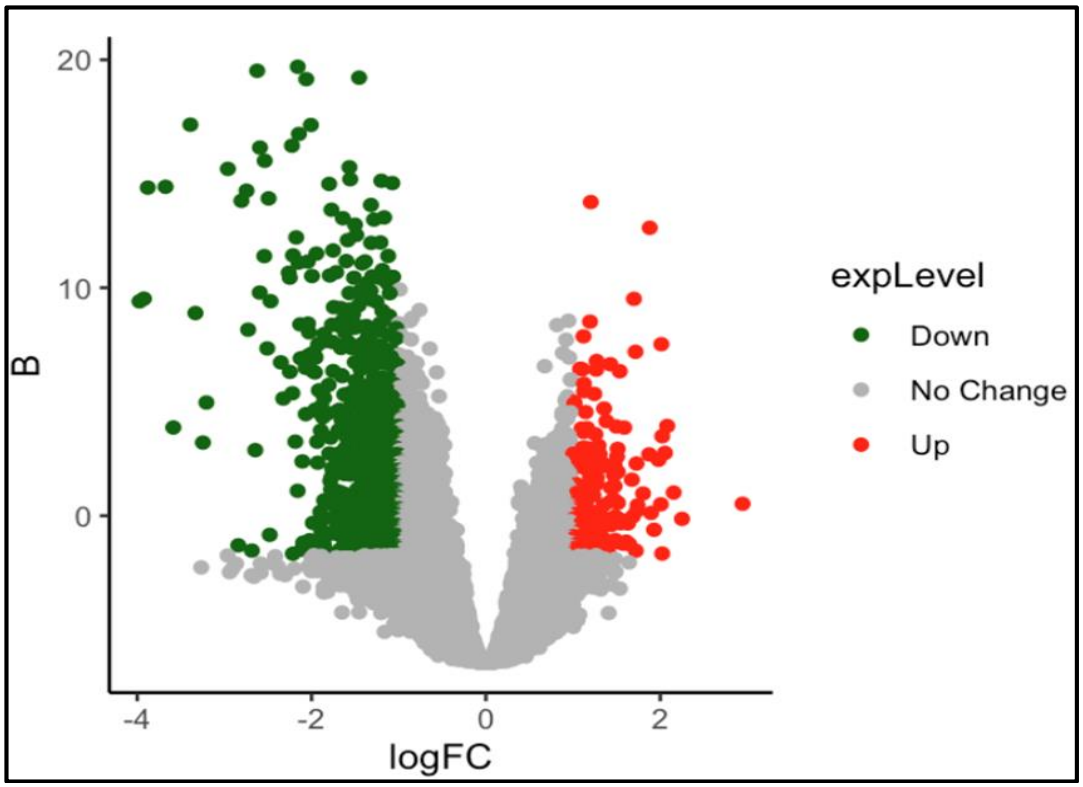

Figure 2: DEGs in Breast Cancer: Utilizing Volcano Plot Analysis to contrast DEGs between Triple-Negative Breast Cancer (TNBC) and non-TNBC Subtypes. The red, green and grey colours indicate the upregulated, downregulated and insignificant levels of gene expression

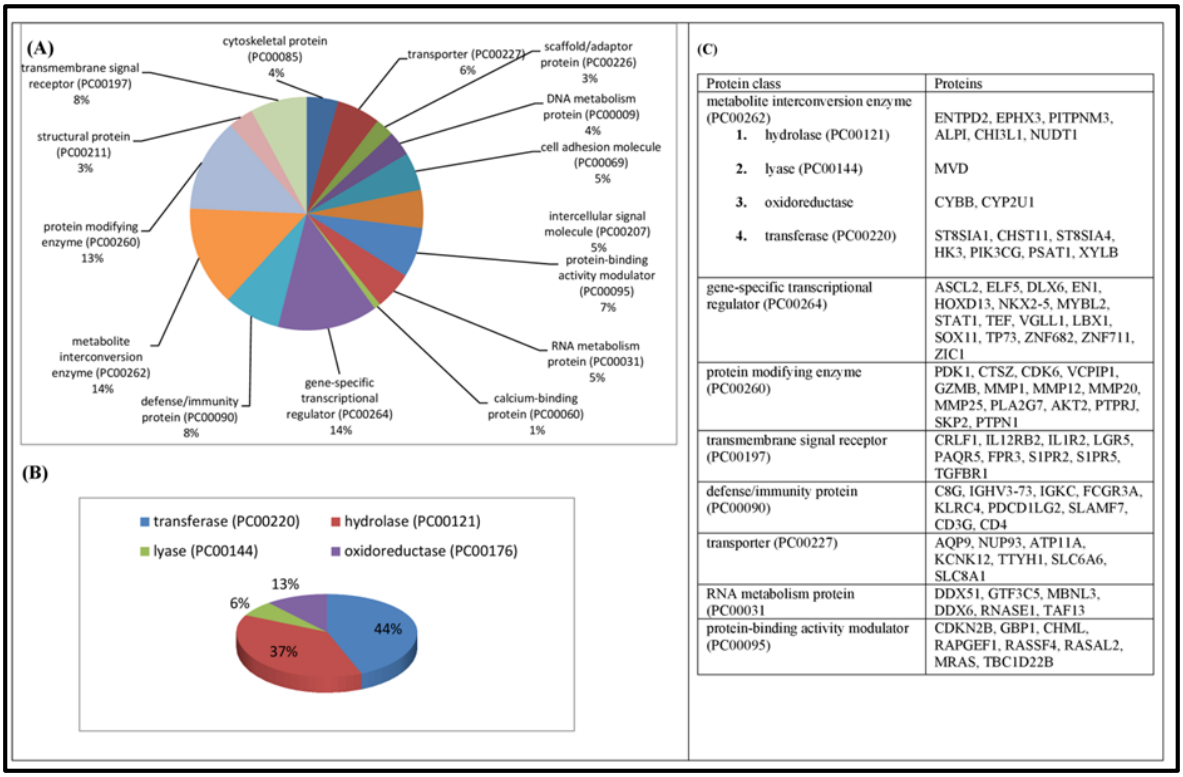

Figure 3: Differentially Expressed Genes (DEGs) are classified according to their protein class, with each protein class identified by its Panther protein class ID. Pie chart showing the distribution of genes in different protein classes

ST8 alpha-N-acetyl-neuraminide alpha-2, 8-sialyltransferase 1 (ST8SIA1) is known to play key role in chemoresistance development in TNBC cells. In a study, Wan et al[37] noticed that ST8SIA1 expression is upregulated at both the mRNA and protein levels, which can contribute to the survival and proliferation of chemoresistant cells. Inhibition of ST8SIA1 improves the effectiveness of chemotherapy by blocking the FAK/Akt/Mtor and Wnt/β-catenin signalling pathways, this suggests that targeting ST8SIA1 could be a potential therapeutic strategy to overcoming chemoresistance in TNBC[51]. In a comprehensive transcriptomic analysis study using RNA sequencing data from TCGA, Kan et al[20], revealed that ST8SIA1 plays a pivotal role in poor overall survival (OS) and disease-free survival (DFS), in BC particularly in TNBC patients[20].

Another study found that ST8SIA1 is having strongly positively correlation with TP53 mutations, which are commonly linked to tumorigenesis and drug resistance. Additionally, ST8SIA1 showed negatively correlation with GATA3 mutations, which are associated with epithelial cell differentiation[4]. Another study demonstrated that inhibiting ST8SIA1 in TNBC cells leads to the depletion of BC stem cells (BCSCs), This effect is achieved by downregulating key signaling pathways including AKT, IL8, STAT3, p53, KRAS, WNT, NFκB, NANOG and RB, while upregulating PTEN and GM1-mediated signaling. These results suggest that ST8SIA1 may be a promising potential target for the management of TNBC[38]. We found significantly increased expression of ST8SIA1 in this study. Claudin-low BC are significantly more aggressive than claudin-high types and they are generally characterized by features such as higher grade, enrichment of stemness characteristics and a tendency to metastasize and they respond poorly to conventional therapies. Inhibition of phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit gamma (PIK3CG) in combination with paclitaxel has shown promising results in addressing this subtype. Combined treatment leads to significant reduction in tumor cell growth, migration and improved apoptosis in both *in vivo* and *in vitro* models[6]. This study also demonstrated the higher expression of PIK3CG.

Phosphoserine aminotransferase 1 (PSAT1) played a crucial role in the synthesis of serine amino acid. Cancer cells rely on large amounts of specific amino acids to survive in nutrient deficient or challenging environments. Serine plays a critical role in cancer cell metabolism by supplying one-carbon units essential for the synthesis of biomolecules including protein, nucleic acid, cofactors and lipids. Its production from the glycolytic intermediate 3-phosphoglycerate (3PG) is particularly important for cancer cell proliferation, especially in TNBC. Serine deficiency reduces TNBC cell growth. Depletion of serine significantly inhibits TNBC cell growth[43].

Results from a study indicate that the expression of PSAT1 increased with the clinical grade of TNBC, additionally the suppression of PSAT1 significantly impacted the dynamics and organization of the cytoskeleton particularly in TNBC cells[35]. We also absorbed the highly expression of PSAT1in our study.

Increased expression of Achete scute-like 2 (ASCL2) has been associated with various role in BC including enhanced growth and size of tumor cells. Faramarzi et al[11] identified ASCL2 players in the Wnt signaling pathway. They have demonstrated that knockdown ASCL2 disrupts the expression of Wnt-associated genes such as survivin (BIRC5) and CD44, ultimately leading to reduced cellular migration. We have found the higher expression of ASCL2 in TNBC cases as compared to Non-TNBC.

Gene set/pathway enrichment analysis (PEA): Gene set/pathway enrichment analysis (PEA) identified DEGs that may be linked to various cellular processes. These DEGs exhibit coordinated functional relationship with key biological processes, cellular components and molecular functions. In this study we identified several enriched pathways including natural killer cell mediated immunity, natural killer cell mediated cytotoxicity (p.adjust ≈ 0.001, purple colour), ensheathment of neurons, axon ensheathment, myelination (p.adjust ≈ 0.003, blue colour), as well as chromosome segregation (p.adjust ≈ 0.002 purple colour). These pathways were associated with key biological process and cellular component and our finding indicates that they are highly activated with statistically significant values. Additionally, several other pathways including xenobiotic catabolic process, cytosolic small ribosomal subunit, cell-cell signaling and multiple pathways related to the extracellular environment, were found to be suppressed in these results.

Pathways associated gene in TNBC: The neural environment surrounding tumors plays detrimental role in cancer progression, promoting tumor growth and metastasis through nerve-tumor interactions, primarily mediated by neurotransmitters and neurotrophic factors. Targeting and disrupting these neural elements may offer a potential strategy to slows cancer progression. A study found that sensory neurons are abundant in TNBC and their interaction promotes cancer cell growth. Co-culture experiments showed that TNBC cells adhered to sensory neuron fibers, significantly increasing their migration rate. Gene expression analysis also further revealed that sensory nerves upregulated genes involved in cancer cell migration and adhesion[22]. In this study, we identified several neutrons activated genes including UGT8, AKT2, BCAS1, PMP22 and JAM3, which may be involved in pathways related to ensheathment of neurons, axon ensheathment and myelination.

Chromosomal instability (CIN) is a result of errors in chromosome segregation during mitosis. CIN plays a central role in cancer progression by driving tumor development through genomic alterations and inflammatory signaling. It not only generates genetic heterogeneity but also promotes immune evasion. Furthermore CIN has been associated with poor prognosis, metastasis and resistance to therapy in cancer[2]. Several studies have highlighted that the primary cause of CIN in BC is the disruption of chromosomal segregation. This disruption arises from various factors including centrosomal aberrations, instability in kinetochore-microtubule attachment, over activation of spindle assembly checkpoint (SAC) and dysfunction in chromatid condensation[27].

In another study, it was observed that increased expression of spindle assembly checkpoint-related genes MAD1, BUB1B, TTK, CDC20, TRIP13, MAD2L1 and BUB1 over-activates the checkpoint in BC, leading to mitotic slippage, which ultimately promotes CIN and contributes to cancer progression.[9]

In our study, we also identified chromosome-associated activated genes including MYBL2, KIF23, BRIP1, CENPN, KIFC1, XRCC3 and CDCA8, which may be associated with CIN, leading to TNBC development. Various studies have demonstrated that certain pathways and downregulated genes associated with biological processes play a crucial role in cancer progression.

Similarly, in our study, we identified a xenobiotic suppressed catabolic process, which may be involved in biological processes of cancer progression. Xenobiotics are foreign chemicals to normal metabolic processes; if they are not metabolized, they can accumulate to toxic levels in the body[55]. In a study, Naushad and colleagues[37] observed that the disruption of xenobiotic metabolism, along with impaired one-carbon metabolism, leads to increased oxidative DNA damage and a higher risk of BC. They also identified specific genetic variants in xenobiotic-metabolizing enzymes, such as CYP1A1, GST and COMT, that are associated with elevated risk of BC. In our study, we found that the genes GSTM1, CYP2A6, GSTM2, CYP2B6 and GSTM3, may be associated with the xenobiotic catabolic process.

DEGs associated with various biological functions: Gene ontology analysis revealed that many genes are associated with diverse active biological pathways including natural killer (NK) cell-mediated immunity, cytotoxicity, cell killing, ensheathment of neurons, axon ensheathment, myelination and chromosomal segregtion, all of which play important roles in cancer biology. However, considering the fundamental importance of chromosome segregation in maintaining genomic stability and its potential implications in tumor progression, this study mainly focuses on genes involved in this pathway to explore their mechanistic roles and therapeutic relevance.

DEGs associated with natural killer cell mediated immunity, cytotoxicity and cell killing: Natural killer cells are essential components of the immune system, playing a crucial role in protecting the body. They are especially important for managing infections and conducting cancer surveillance. TNBC cells have a unique subpopulation of NK cells characterized as Socs3highCD11b−CD27− immature NK cells, which exhibit pro-tumorigenic

properties. These NK cells reduce cytotoxicity while simultaneously activating cancer stem cells, thereby enhancing tumor progression through Wnt signaling. The study suggest that targeting these NK cells may improve the outcomes for patients with TNBC[48]. In this study we identified DEGs associated with natural killer cells mediated immunity and cytotoxicity that included NKG7, SLAMF7, KLRC4, GZMB and FCGR3A.

Natural killer cell granule protein (NKG7) is a key molecule for the effective cytotoxic function of CD8+ T cells. Downregulation of NKG7 is linked to poor response to immune checkpoint inhibitors and reduces T-cell mediated cytotoxicity. In a study by Wen et al[57], it was observed that restoring NKG7 expression enhanced antitumor activity, suggesting that NKG7 could be a promising target in cancer immunotherapy. Signaling lymphocytic activation molecule F7 (SLAMF7) is strongly correlated with clinicopathological factors in BC. Overexpression of SLAMF7 is linked to improved disease-free and disease-specific survival. While low expression is directly associated with poor survival of BC patients. Wang et al[55,56] conducted a study and found that N-linked glycosylation of SLAMF7 promotes BC, with seven glycosylation sites identified, particularly highlighting the importance N98. They used a small molecule inhibitor (NGI-1) to block glycosylation, which enhanced antibody affinity. The study concluded that deglycosylation of SLAMF7 could serve as potential strategy in cancer immunotherapy.

In a study, GO enrichment analysis revealed that the upregulated genes in the immune group are potentially involved in T cell activation, plasma membrane externalization and receptor-ligand interaction. Notably many of these upregulated genes, such as CD226 and KLRC4-KLRK1, are associated with improved overall survival in specific stages and subtypes of BC. These findings suggest that CD226 and KLRC4-KLRK1 may be promising candidates for targeted immunotherapy, offering potential strategies for BC management that could enhanced treatment outcomes and survival rates[47].

Fiscon et al[12] conducted a gene expression network-based analysis on TNBC using data from the TCGA database. Their results identified HMGA1, FOXM1 and MYBL2 as key switch genes. These genes are not only involved in TNBC development but also regulate each other's expression. The study suggested that these genes could serve as potential therapeutic targets in TNBC. In another study, it was observed that PITPNA-AS, a non-coding RNA activated by MYBL2, plays an oncogenic role in TNBC. It upregulates SIK2 by sponging miR-520d-5p and recruiting DDX54 protein[30]. This gene was linked to chromosomes segregation (Figure 4).

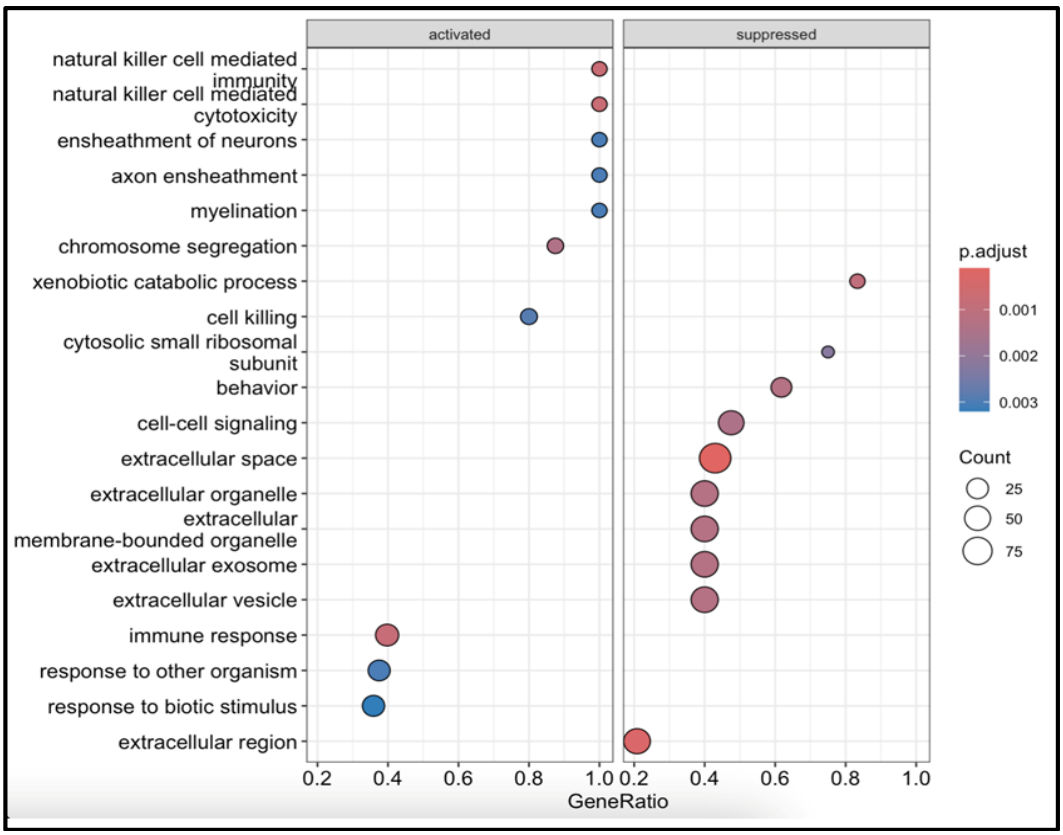

Figure 4: Gene Ontology (GO) analyses of DEGs between TNBC and non-TNBC. A variety of deregulated enzymes have been linked to the pathogenesis of triple-negative breast cancer (TNBC), size of the circle indicates the number of genes within a category, the color of the circle indicates the adjusted p-value from the enrichment analysis: a smaller p-value is shown with higher red color intensity, while a larger p-value is indicated with higher blue color intensity

DEGs associated with chromosome segregation pathway: Here, we identified the genes such as MYBL2, KIF23, BRIP1, CENPN, KIFC1, XRCC3 and CDCA8 which are related to chromosome segregation pathway. Kinesin family member 23 (KIF23) is significantly overexpressed in TNBC. Research by Jian et al[19], demonstrated that knockdown of KIF23 via miRNA in TNBC cell lines (MDA-MB-231 and BT549) inhibits epithelial-mesenchymal transition (EMT). This effect is specifically mediated by miR-195-5p, which binds to the 3' translated region (3'UTR) of KIF23, promoting its degradation and there by suppressing EMT. Similarly, another study found that elevated KIF23 expression is associated with TNBC both *in vitro* and *in vivo*.

The transcription of KIF23 is regulated by FOXM1, which is upregulated by WDR5 through H3K4me3 modification. FOXM1binds to the promoter region of KIF23, promoting the progression of TNBC via the Wnt/β-catenin pathway. These finding suggests that targeting the WDR5/FOXM1/ KIF23/Wnt/β-catenin axis could be a promising therapeutic strategy for TNBC[26].

BRCA1 interacting helicase 1 (BRIP1) is overexpressed in luminal BC compared to TNBC. Kaplan–Meier analysis indicates that its overexpression is linked to poor survival outcome. Additionally, studies reveled that BRIP1 exhibits dual functionality, acting as both an oncogene and tumor suppressor. A recent study emphasized that BRIP1 is a novel contributor to BC, particularly in cell proliferation, migration and invasion. While its role in DNA damage repair is well established, its elevated expression in BC further supports its involvement malignant phenotype. siRNA mediated downregulate of BRIP1 reveals its critical role in cancer regulation. These findings suggest that BRIP1 could serve as a potential biomarker and therapeutic target for BC management[41].

Centromere protein N (CENPN) is expressed in several cancers including BC and is frequently co-expressed with immune checkpoint-related genes. Study shows that depletion of CENPN inhibits BC migration and proliferation. These finding suggest that CENP may be a promising target for immune checkpoint inhibitors [14]. A similar study revealed that the upregulation of CENPN is linked to poor survival outcomes in BC patients. The underlying mechanism involves CENPN promoting cell proliferation and enhancing aerobic glycolysis through activation of AKT/HIF-1a signaling pathway. Consistent with prior findings, this research supports the potential of targeting CENPN for cancer therapy[56]. Kinesin family member C1 (KIFC1) plays a crucial role in centrosome clustering, a process vital for the survival of cancer cells. Its expression is significantly elevated in TNBC compared to normal cells.

In a study by Li et al[26], the small molecule inhibitor PJ34 was found to be highly effective in inhibiting KIFC1. These finding highlighted KIFC1 as a promising therapeutic target for BC the management. Ogden et al[40], conducted a study on nuclear KIFC1 (nKIFC1) and found it to be a strong prognostic marker for poor outcomes in African American (AA) TNBC patients compared to white patients. Overexpression of nKIFC1 was associated with worse overall survival, distant metastasis-free survival and progression-free survival in AA patients. Furthermore, silencing nKIFC1 significantly inhibited cell migration in AA TNBC cells.

Similarly, another study found that nKIFC1 plays crucial in TNBC progression and metastasis in AA patients compared to European American (EA) patients. Mechanistically, nKIFC1 interacts with the tumour suppressor myosin heavy chain 9 (MYH9) to drive this process. Disrupting the interaction between nKIFC1 and MYH9 could significantly reduce cell proliferation, migration and invasion[13]. These finding suggest that nKIFC1 could be a crucial therapeutic target for AA TNBC Patients.

MYB proto-oncogene-like 2 (MYBL2) has a critical role in regulating cellular differentiation and cell proliferation in BC. A study confirmed that MYBL2 is highly expressed in breast cancer cells and is regulated by miR-143-3p, which targets its 3′-UTR and inhibits its expression. In addition, MYBL2 inhibits apoptosis, which emphasizes its importance in tumour progression. However, further research is needed to fully understand the underlying mechanisms of MYBL2 and its potential as a therapeutic target in breast cancer[8]. In a study, Hu et al[16] highlighted the critical role of XRCC3 in BC development. XRCC3 is essential for MCF7 cell proliferation and XRCC3 downregulation leads to DNA damage accumulation and p53-dependent cell death. Conversely, elevated XRCC3 expression increases tutor aggressiveness. Additionally, they suggest a significant relationship between XRCC3 and RAD51 expression levels and HER2 status, a known poor prognostic factor in BC.

Furthermore, overexpression of XRCC3 correlates with larger tumor size, whereas elevated RAD51 levels are associated with axillary lymph node metastasis. Based on these findings, they proposed that XRCC3 may play important roles in breast cancer pathogenesis, making them potential targets for further research and therapeutic intervention[16].

A study conducted by Bu et al[5] found that CDCA8 has been identified as a crucial mediator of estrogen-stimulated BC cell growth and survival. Its expression is increased in E2-stimulated MCF7 and T47D cells as well as in tumor samples. Knockdown of CDCA8 impairs cell survival and growth, arrests G1 phase and decreases the expression of E2-induced molecules (cyclin D1 and BCL2), while increasing apoptosis-related markers (P21 and P27). In addition, using Kaplan-Meier analysis demonstrated that high CDCA8 expression is associated with poor prognosis. These findings

suggest that CDCA8 may serve as a potential therapeutic target for estrogen-driven breast cancer (Figure 4).

Receiver operating characteristic (ROC) analysis: ROC analysis was conducted to evaluate the performance of multiple gene expressions in distinguishing between non-TNBC and TNBC samples. The ROC curves for seven genes, namely KIF23, CENPN, MYBL2, CDCA8, KIFC1, BRIP1 and XRCC3, were plotted and their area under the curve (AUC) values were calculated to assess their discriminatory power.

Among the genes analysed, KIF23 exhibited the highest AUC value of 0.79, indicating its strong potential as a biomarker for accurately identifying TNBC samples. This was closely followed by BRIP1 with an AUC of 0.77, suggesting that it also has a significant capability in distinguishing TNBC cases. Other genes, such as CENPN, MYBL2, CDCA8, KIFC1 and XRCC3, demonstrated lower AUC values of 0.73, 0.74, 0.65, 0.66 and 0.74, respectively, indicating lesser performance in comparison to KIF23 and BRIP1 (Figure 5).

The ROC curves and corresponding AUC values underscore the potential utility of KIF23 and BRIP1 as reliable biomarkers for TNBC classification. The analysis highlights the importance of these gene expressions in enhancing the accuracy of TNBC diagnosis, facilitating targeted treatments and ultimately improving patient outcomes in BC management. This analysis provides valuable insights into the molecular profiling of TNBC and non-TNBC samples, paving the way for personalized medicine approaches in oncology.

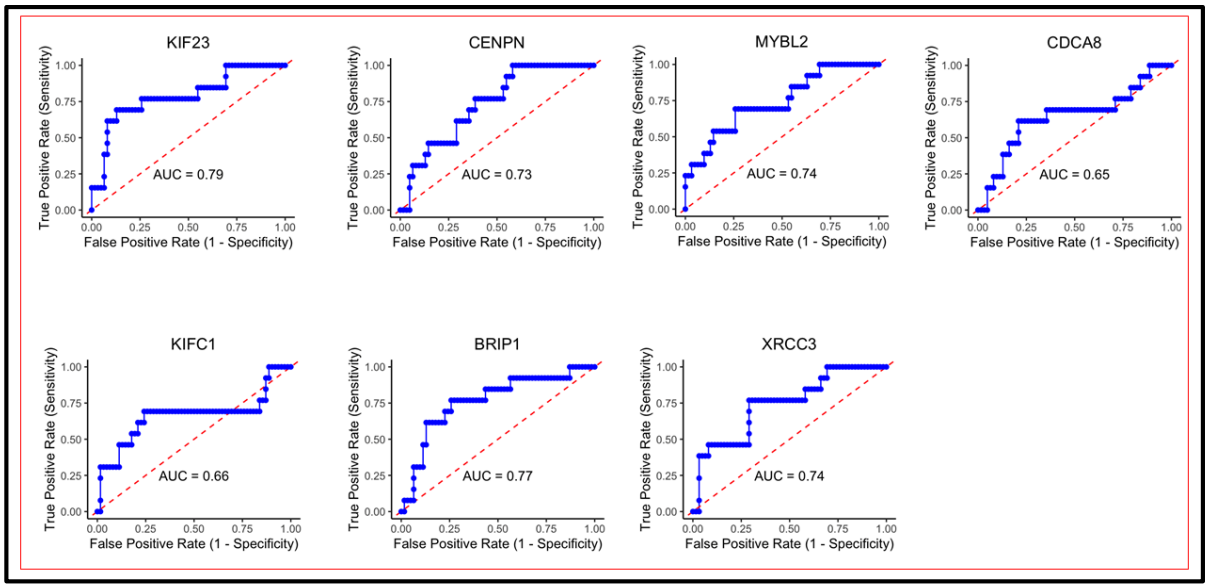

Figure 5: ROC Plots depict highly variable genes found to be deregulated genes in TNBC in comparison to non-TNBC samples. The AUC values illustrate the varying effectiveness of these genes in identifying TNBC samples, with KIF23 and BRIP1 showing the highest potential as biomarkers in this context

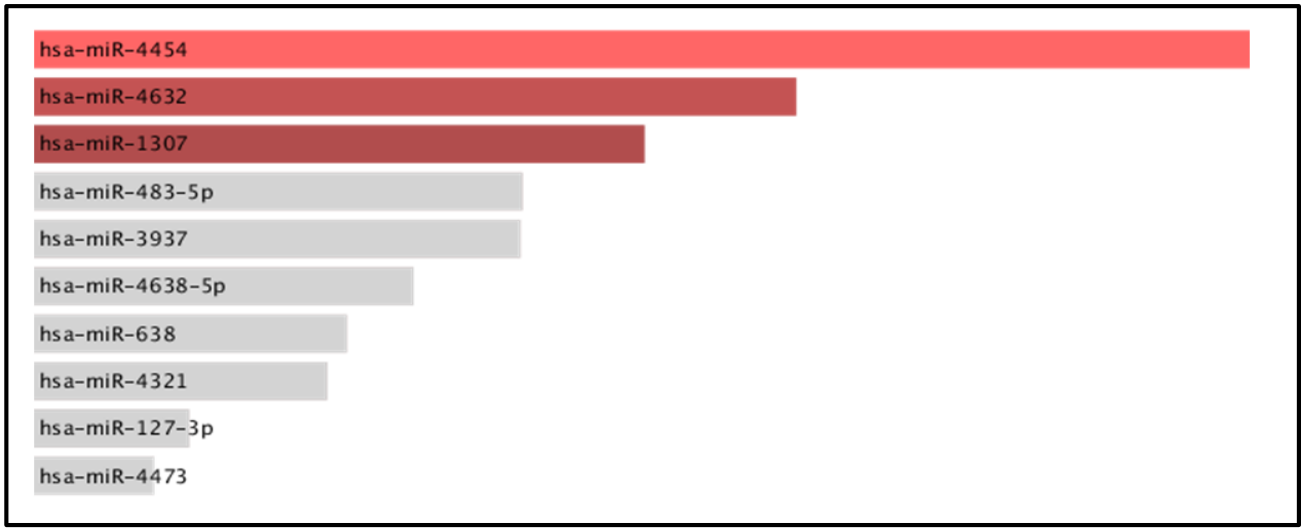

Figure 6: Bar graph showing miRNAs targeting genes ranked p-value. Dark red bar represents statically significant miRNAs ($p < 0.05$), While grey bars indicate miRNAs that are not statistically significant

Table 2
Enriched miRNAs and their associated target genes

miRNA	P-value	Odds Ratio	Combined Score	Genes
hsa-miR-4454	0.015899536	13.48402778	55.84363423	BRIP1;CENPN
hsa-miR-4632	0.033077534	8.997414806	30.67129584	MYBL2;CENPN
hsa-miR-1307	0.042260006	7.827572016	24.76576576	BRIP1;CENPN

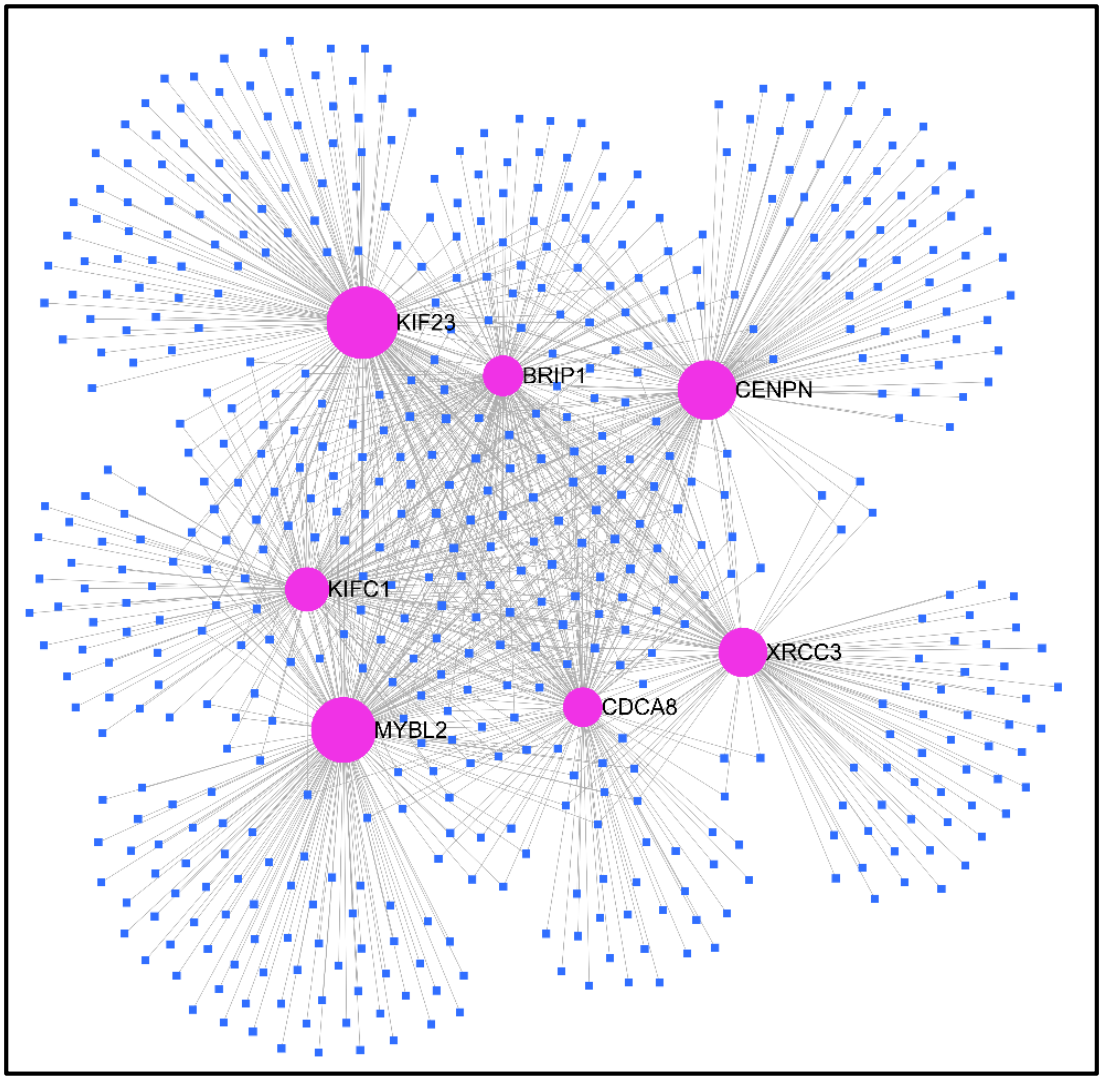

Figure 7: Network of identified miRNAs and their target genes made by miRNet 2.0. The round pink spots represent DEGs and the blue colored squares represent the miRNAs

MicroRNA binding to chromosomal segregation related genes: Enrichr web tools were utilized to identify miRNAs associated with chromosomal segregation-related genes, 214 miRNAs were identified that regulate MYBL2, KIF23, BRIP1, CENPN, KIFC1, XRCC3 and CDCA8 genes. Among these miRNAs (statistically significant ($p < 0.05$)) namely miR-4454 and miR-1307 regulate BRIP1 and CENPN genes, while miR-4632 regulate MYBL2 and CENPN genes (Table 2 and figure 6). Furthermore, the miRNAs regulate KIF23, KIFC1, XRCC3 and CDCA8 genes. These miRNAs play significant role in BC initiation and progression. miR-1307-3p significantly increased in early stage of BC tissues and promotes proliferation, invasion and supports the formation of new blood vessels by repressing the tumour suppressor protein MYND domain-containing protein 4 (SMYD4)[10,15].

Additionally, miR-1307 also contributes to cisplatin resistance in BC by regulating apoptosis via targeting Mdm4[53]. miR-4454 has role in cell proliferation, migration, invasion and vascularization in hepatocellular carcinoma (HCC) cells by targeting the vacuolar protein sorting 4 homolog A (Vps4A) and Rab27A proteins. In their study, Lin et al[29] noticed that downregulation miR-4454 leads to cell cycle arrest, increased apoptosis and ROS production in these cells. They also suggested that targeting this miRNA could significantly improve outcomes for hepatocellular carcinoma. miR-4632 has critical role in the regulation and

proliferation of human pulmonary artery smooth muscle cells (HPASMCs) by targeting cJUN. Its expression is significantly reduced in response to platelet-derived growth factor-BB (PDGF-BB) stimulation, that was associated with histone deacetylation via the PDGF receptor/PI3K/HDAC4 signalling pathway.

miRNA may be a potential therapeutic target for pulmonary vascular remodeling diseases. Web tool miRNet v2.0 (https://www.mirnet.ca/) was used for miRNA-mRNA interaction mapping, which revealed that genes associated with chromosomal segregation have strong interactions with miRNAs. Among them, MYBL2, KIF23, CENPN and XRCC3 showed the highest degree of interaction while KIFC1, BRIP1 and CDCA8 showed the least level of interaction (Figure 7).

Conclusion

The mortality rate of TNBC is very high as compared to other BC subtypes. The effective treatments for TNBC has been challenging for patients and clinicians due to poor prognosis and heterogeneity. In this study, we utilized transcriptome data and identified several genes and associated pathways that play crucial role in TNBC. We identified 998 DEGs in TNBC as compared to non-TNBC. Of these, 169 were upregulated and 829 were downregulated. These genes predominantly belong to the protein classes such as metabolite interconversion enzymes and gene-specific transcriptional regulators.

Gene set/pathway enrichment analysis revealed that MYBL2, KIF23, BRIP1, CENPN, KIFC1, XRCC3 and CDCA8 are associated with the highly activated chromosome segregation pathways, while regulatory analysis identified miR-4454, miR-4632 and miR-1307 potentially modulating the expression of MYBL2, BRIP1 and CENPN genes. ROC analysis demonstrated that KIF23 and BRIP1 exhibited the highest AUC values (0.79 and 0.77 respectively), indicating their strong potential as biomarkers for TNBC detection. However, validation of both genes under *in vivo* and *in vitro* conditions are very important to find their involvement in TNBC.

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