



Identification and analysis of dysregulated fatty acid metabolism genes in breast cancer subtypes

Umar Yousuf¹ · Shazia Sofi¹ · Aanisa Makhdoomi¹ · Manzoor Ahmad Mir¹ 

Received: 20 August 2022 / Accepted: 22 September 2022 / Published online: 12 October 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract

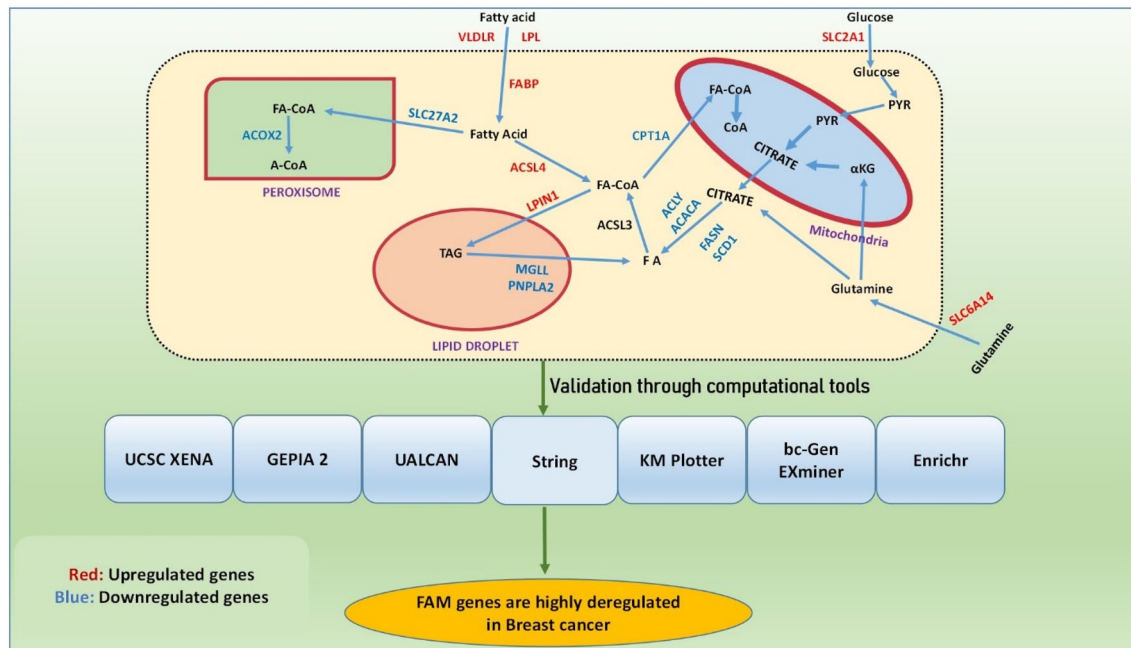
Breast cancer is one of the most aggressive and lethal types of transformation among women. An anomaly of normal fatty acid metabolism is acknowledged as a critical trigger for malignant transformations including breast cancer, but the prospect of targeting fatty acid metabolism for the treatment of malignancy has remained unrecognized so far. It has been observed that specific fatty acid metabolism genes are involved in the commencement and development of breast cancer. These specific genes have also been observed to be related to different isotypes/molecular subtypes of breast cancer. The main purpose of this study was to scrutinize the prognostic significance, functional role, and expression pattern of fatty acid metabolism genes. In-Silico tools like TCGA BrCA, Gepia2, Ualcan Analysis, UCSC Xena, Kaplan–Meier plotter, Bc-gene EXminer, String, gene ontology, and KEGG databases, were used to assess the expression pattern of the fatty acid metabolism genes in breast cancer patients and also among the different molecular sub-types of breast cancer. Differential gene expression analysis revealed dysregulation of FABP4, FABP5, PLIN1, PLIN2, PLIN4, PLIN5, LPIN1, MGLL, PNPLA2, PNPLA7, ACSL1, and ACOX2 showing a fold change $> \pm 1.5$. Also, most of these genes show downregulation in Ualcan analysis of different isotypes/molecular subtypes of breast cancer. The study reveals that the screened genes i.e., FABP4, FABP5, PLIN1, PLIN2, PLIN4, PLIN5, LPIN1, MGLL, PNPLA2, PNPLA7, ACSL1, and ACOX2 can be used as biomarkers that reveal poor prognosis and may serve as therapeutic targets for the treatment of breast cancer.

Umar Yousuf and Shazia Sofi have contributed equally to this work.

✉ Manzoor Ahmad Mir
drmanzoor@kashmiruniversity.ac.in

¹ Department of Bioresources, School of Biological Sciences,
University of Kashmir, Srinagar, J&K 190006, India

Graphical abstract



Keywords Breast cancer · Dysregulated genes · Fatty acid metabolism · Bioinformatics · Prognosis · Gene ontology · Survival probability · Expression pattern

Abbreviations

RFS	Relapse free survival
OS	Overall survival
GO	Gene ontology
BC	Breast cancer
KEGG	Kyoto Encyclopaedia of Genes and Genomes
TCGA	The Cancer Genome Atlas
MF	Molecular function
KM	Kaplan Meier
ER	Estrogen
PR	Progesterone
HER2	Human epidermal growth factor receptor 2
EGFR	Epidermal growth factor receptor
TNBC	Triple negative breast cancer
BP	Biological processes
CC	Cellular compartment
SBR	Scarff Bloom Richardson
PPAR	Peroxisome proliferator-activated receptors
FA	Fatty acid
PYR	Pyruvate
SLC2A1	Solute carrier family 2 member (GLUT1)
FABP	Fatty acid binding protein
αKG	Alpha ketoglutarate
LPL	Lipoprotein lipase
ACOX2	Acyl-CoA oxidase

FA-CoA	Fatty actyl CoA
A-CoA	Acetyl CoA
TAG	Triacyl glycerides

Introduction

Breast cancer is the most common cancer in female individuals responsible for the high rate of malignancy [1–3]. Excluding basal cell and squamous cell skin cancers, breast cancer is the most commonly diagnosed cancer among women in the U.S. In 2022, there will be an estimated 287,850 new cases of invasive breast cancer diagnosed in women; 2710 cases diagnosed in men, and an additional 51,400 cases of ductal carcinoma in situ (DCIS) diagnosed in women. [4, 5]. Breast cancer is a heterogeneous solid tumor with > 20 distinct subgroups differing in appearance, clinical behavior, and genetics [6]. Breast cancer has been categorized into triple-negative breast cancer (TNBC), luminal A, luminal B, & HER2+ based on gene expression [7, 8]. Luminal A, the most common subtype, may be PR positive, ER positive or negative, HER2 negative, and EGFR and CK5/6 negative [9–12]. Lower expression of progesterone receptor (PR), estrogen receptor (ER), and high histologic grade are characteristics of luminal B tumors [13].

HER2+ subtype may be ER/PR negative, HER2 positive, and EGFR and CK5/6 negative [9, 10]. TNBC is the most aggressive type of breast cancer which accounts for nearly 20% of identified breast cancer cases & is characterized by the absence of progesterone (PR) receptor, estrogen receptor, and or under-expression of human epidermal growth factor receptor 2 (HER2) [13–15]. Among breast cancer isotypes, TNBC has very poor overall survival and enhanced metastatic potential [16–21].

To develop and survive, cancer cells frequently alter the human body's metabolism [22]. One of the hallmarks of tumor cells that are currently being realized is any abnormal change in fatty acid metabolism [11, 23–27]. The modification of fatty acid metabolism, necessary to achieve the high rate of fatty acid synthesis for cancer cell proliferation is one of the most intriguing modifications. Therefore, fatty acid metabolism needs to be reprogrammed for cancer cells to survive. Since it can alter cellular processes, fatty acid metabolic reprogramming is one of the characteristics of a cancer situation. Increased exogenous fatty acid intake, de novo fatty acid synthesis and fatty acid oxidation are all components of the reprogramming of fatty acid synthesis. For diagnosing and estimating the effectiveness of the therapy regimen for treating tumors, it is helpful to identify the biochemical targets in fatty acid metabolism. [28–30]. This dependency of cancerous cells on the altered fatty acid metabolism reveals that the proteins involved in this process may prove good therapeutic targets for the treatment of cancer [31–33].

Dysregulation of fatty acid metabolism is recognized as a component of malignant transformation in many different cancers, including breast; yet the potential for targeting this pathway for prevention and/or treatment of cancer remains unrealized. Evidence indicates that proteins involved in both the synthesis and oxidation of fatty acids play a pivotal role in the proliferation, migration, and invasion of breast cancer cells [25]. Thus targeting the fatty acid metabolism pathway and the genes related to it will prove an efficient treatment strategy for breast cancer patients. In this study, we evaluated the expression of fatty acid metabolism genes using various bioinformatic tools. We used the TCGA BRCA database obtainable from Gepia2 and UCSC XENA web servers to evaluate the expression of fatty acid metabolism genes in clinical samples of BC patients. Here we came up with the significant dysregulation of fatty acid metabolism gene expression in breast cancer patients. Furthermore, this dysregulated gene expression was also found to exert an influence on relapse-free survival (RFS) and overall survival (OS) in breast cancer patients. Our findings suggest that fatty acid metabolism gene expression is downregulated in different tumor types and normal activation of their expression may reduce abnormal cell proliferation and may give better treatment outcomes in certain cancers.

Results

Expression of fatty acid metabolism genes is dysregulated in breast cancer

The gene expression pattern of fatty acid metabolism was examined in BC using UCSC XENA. Fatty acid metabolism gene expression analysis shown by the TCGA BrCa database disclosed a notable gene dysregulation in BC (Fig. 1). Additional analysis of different fatty acid metabolism genes obtained from the Gepia2 dataset displayed a log₂-fold change as shown in Table 1. FABP4, FABP5, PLIN1, PLIN2, PLIN4, PLIN5, LPIN1, MGLL, PNPLA2, PNPLA7, ACSL1, and ACOX2 were found to be downregulated (Fig. 2). The expression pattern of highly dysregulated fatty acid metabolism genes among different BC subclasses was analyzed using UALCAN, and it was found that the Luminal breast cancer patients show high expression of genes PLIN5, MGLL, PNPLA2, and ACOX2 as compared to their expression in TNBC patients. Further, luminal patients show a high expression of ACOX2 as compared to normal. Moreover, it is found that HER2 Positive patients show a very low expression of FABP5, PLIN1, PLIN2, PLIN5, ACSL1, MGLL, PNPLA2, and ACOX2 genes when compared to their normal levels. Adding to this, the expression of PLIN1, PLIN5, ACSL1, MGLL, PNPLA2, and ACOX2 is downregulated in TNBC patients when compared to normal levels (Fig. 3).

Dysregulation of fatty acid metabolism gene is associated with poor prognosis in BC patients

The Kaplan–Meier plotter, a bioinformatic tool for gene expression pattern information & survival probability of BC patients, was used to look over the survival time of highly dysregulated fatty acid metabolism genes. Based on median expression, BC patients were splitted into two groups viz: high expression group and low expression group. The relapse-free survival (RFS) examination was executed on 4929 patients with breast cancer for about 300 months, and it was reported that the patients with low expression of PLIN1, MGLL, ACOX2 have worse RFS than patients with increased levels of FABP5 mRNA, having log-rank *p* value 1.3e-06, Hr-value 0.78 for PLIN1, *p* value 0.1, HR-value 0.88 for MGLL, *p* value 1.6e-08, HR 0.75 for ACOX2, While as, patients with high expression of SLC27A6 and FABP5 show poor RFS with *p* value 0.17 and 1.8 e-13 respectively, Fig. 4A. The tie-up between gene expression of fatty acid metabolism genes and overall survival (OS) was scrutinized in 1879 patients, and it

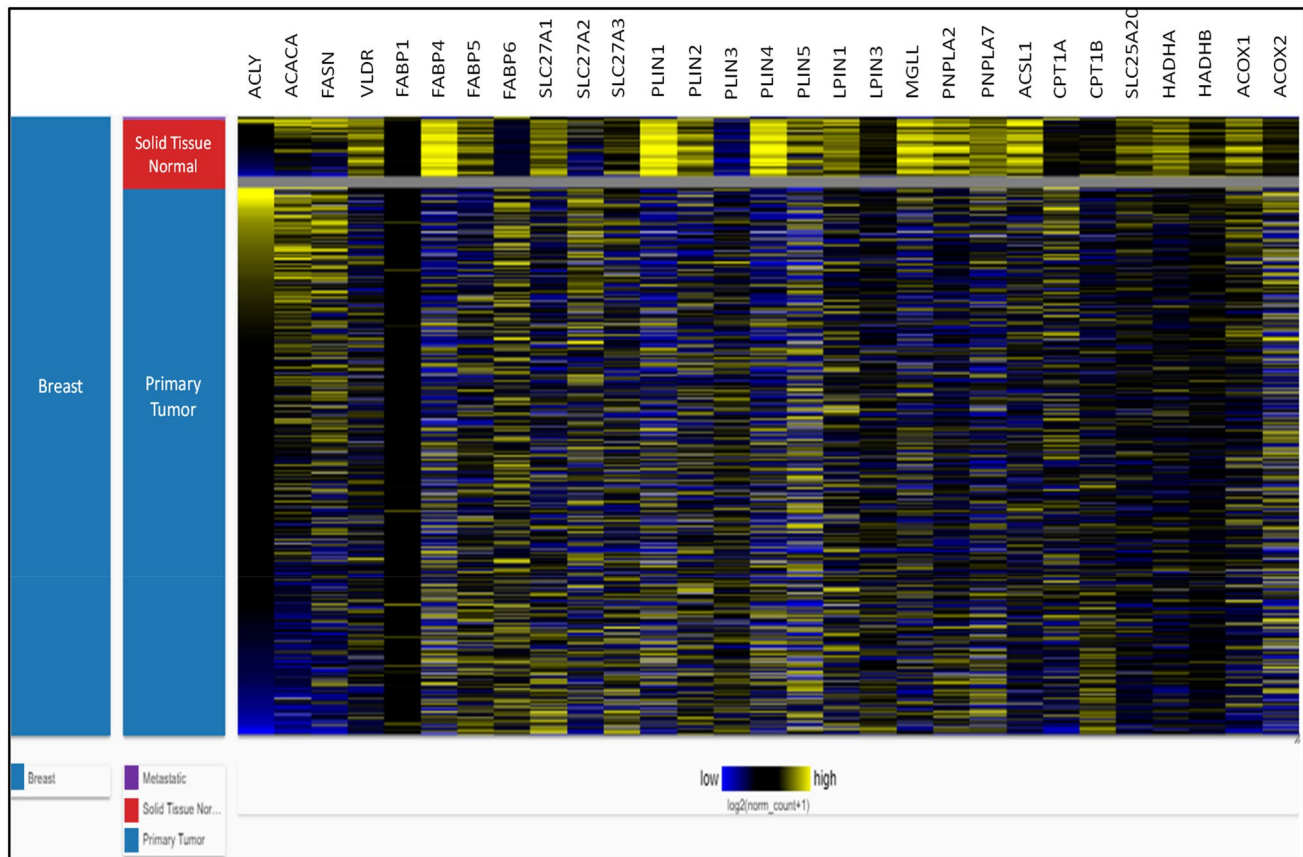


Fig. 1 mRNA expression pattern of Fatty acid metabolism genes in breast cancer patients. Heat map showing the expression patterns of Fatty acid metabolism genes. Gene expression was drawn out using XENA. Contrasting the expression of fatty acid metabolism genes

between GTEx samples (normal breast, $n=291$), Solid Tissue Normal (tissue surrounding the tumor ($n=114$), and primary tumor (cancer, $n=1097$)

was disclosed that patients of breast cancer with increased expression of SLC27A6, and MGLL have a poor OS with p value 0.00058, 0.0024, and HR 1.78, 1.61, respectively, while as patients with low expression of FABP5, PLIN1, PLIN2, PLIN5 PNPLA2 and ACOX2 have worse OS with p value 0.074, 0.03, 0.15, 0.0012, 0.024, 0.029 and HR 0.75, 0.72, 0.79, 0.61, 0.71, 0.81 respectively Fig. 4B.

Correlation of dysregulated fatty acid metabolism genes with clinical-physiological characteristics in BC

The bc-Gen EXminer web source was used to analyze the correlation between highly dysregulated fatty acid metabolism genes and ordinary pathophysiological characteristics in BC patients Fig. 5. FABP4 was shown to exhibit a higher expression in BC patients who expressed hormone receptors (Estrogen and progesterone receptors) (p value <0.0001) and was observed to correlate with SBR grade 1 (p value <0.0001) and individuals with wild p53 had notably increased levels of FABP4 mRNA as compared

to individuals with mutant p53 (p value <0.0001) Fig. 5A. In comparison to FABP4, FABP5 expression levels were notably enhanced in BC patients lacking hormone receptors (Estrogen and progesterone receptors) (p value <0.0001) however did not vary substantially from HER2 (p value of 0.0914). Enhanced FABP5 expression levels were found to be associated with SBR3 (p value <0.0001). Furthermore, patients with mutant p53 had increased levels of FABP5 mRNA than individuals having normal-type of p53 Fig. 5B.

PLIN1 expression levels were high in BC patients who expressed estrogen and progesterone receptors exhibiting p value <0.0001 and was observed to correlate with SBR1 (p value <0.0001). Additionally, individuals having mutant p53 (p value <0.0001) had lower levels of PLIN1 mRNA than those with wild-type p53 Fig. 5C.

PLIN2 expression levels were higher in BC patients deficient in estrogen and progesterone receptors (p value <0.0001). In contrast, PLIN2 expression was greater in HER2-augmented BC (p -value 0.0003). PLIN2 expression was shown to be correlated with SBR3 (p value <0.0001). Moreover, patients with wild p53 had

Table 1 Log2-fold change of Fatty acid metabolism genes in breast cancer

Gene symbol	Gene ID	Median (tumor)	Median (normal)	Log2(fold change)	<i>p</i> value
ACLY	ENSG00000131473.16	66.339	37.859	0.739	2.27e-29
ACACA	ENSG00000278540.4	26.279	18.581	0.478	8.46e-14
FASN	ENSG00000169710.6	249.742	268.577	-0.104	5.06e-4
VLDLR	ENSG00000147852.15	5.280	9.940	-0.801	1.56e-38
FABP1	ENSG00000163586.9	0.000	0.180	-0.239	1.10e-36
FABP4	ENSG00000170323.8	30.290	3082.958	-6.623	8.32e-201
FABP5	ENSG00000164687.10	37.330	132.329	-1.798	2.73e-60
FABP6	ENSG00000170231.15	0.290	0.000	0.367	9.15e-23
SLC27A1	ENSG00000130304.16	12.700	23.840	-0.858	2.63e-77
SLC27A2	ENSG00000140284.10	3.890	0.850	1.402	3.05e-32
SLC27A3	ENSG00000143554.13	33.171	52.308	-0.642	1.53e-36
PLIN1	ENSG00000166819.11	3.230	274.449	-6.025	2.57e-195
PLIN2	ENSG00000147872.9	33.201	168.218	-2.307	2.32e-160
PLIN3	ENSG00000105355.8	51.599	31.130	0.711	1.43e-83
PLIN4	ENSG00000167676.4	2.820	167.729	-5.465	2.65e-203
PLIN5	ENSG00000214456.8	2.530	13.200	-2.008	6.64e-70
LPIN1	ENSG00000134324.11	6.880	22.371	-1.568	8.98e-134
LPIN3	ENSG00000132793.11	6.600	19.631	-1.441	5.89e-109
MGLL	ENSG00000074416.13	20.359	75.279	-1.836	6.03e-108
PNPLA2	ENSG00000177666.15	88.972	389.370	-2.117	1.68e-176
PNPLA7	ENSG00000130653.15	2.900	11.790	-1.713	3.48e-147
ACSL1	ENSG00000151726.13	37.031	161.948	-2.099	1.24e-121
CPT1A	ENSG00000110090	8.570	22.070	-1.269	3.48e-22
CPT1B	ENSG00000205560.12	4.730	11.725	-1.151	2.97e-16
SLC25A20	ENSG00000178537.9	16.080	30.935	-0.903	7.81e-15
HADHA	ENSG000000842754.10	78.962	113.598	-0.519	1.60e-3
HADHB	ENSG00000138029.13	116.379	207.849	-0.831	8.10e-8
ACOX1	ENSG00000161533.11	9.290	17.330	-0.833	9.39e-16
ACOX2	ENSG00000168306.12	3.710	20.779	-2.209	6.34e-14

Bold genes indicate that these genes have shown greater Log2 fold change values as compared to rest of the genes

very low levels of PLIN2 mRNA than those with mutant p53 who have notably higher levels of PLIN2 Fig. 5D.

PLIN4 and PLIN5 expression levels were high in BC patients who expressed estrogen receptor and progesterone receptor (*p* value < 0.0001). Further, it was reported that enhanced PLIN4 and PLIN5 expression was connected with SBR1 (*p* value < 0.0001) and individuals with wild p53 had significantly greater PLIN4 and PLIN5 mRNA levels than patients with mutant-type p53 (*p* value < 0.0001) Fig. 5E, F.

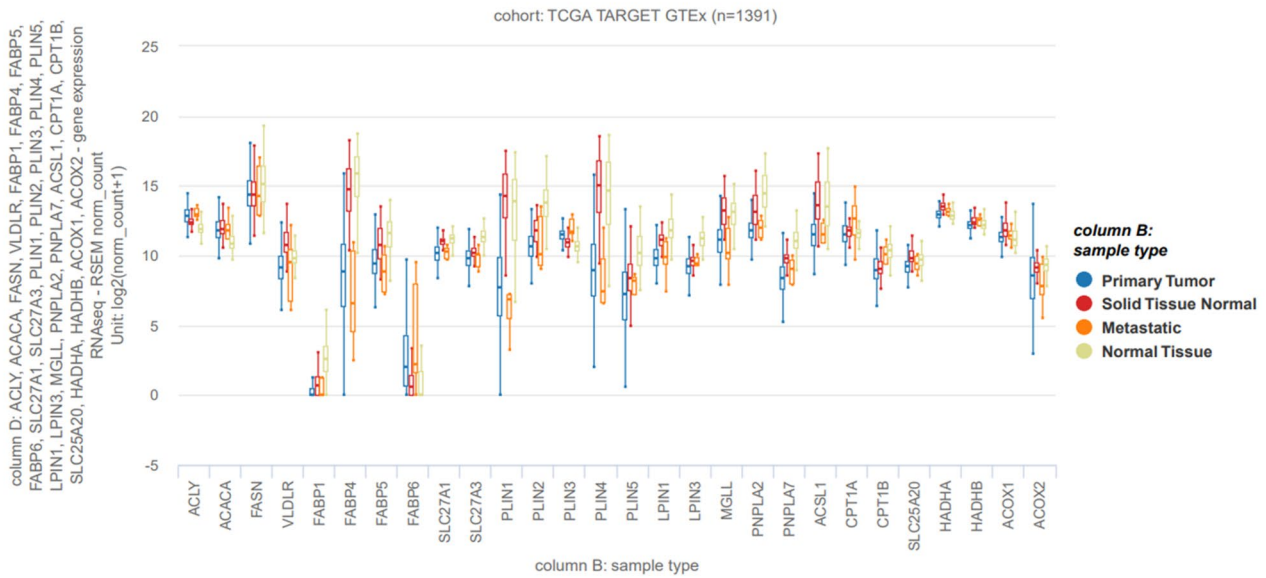
MGLL expression was high in ER+ and HER2 positive patients (*p* value < 0.0001) while as the expression did not vary substantially from PR (*p* value = 0.8046) or SBR grade (*p* value < 0.0001). Further individuals with wild

p53 showed increased levels of MGLL mRNA than those who had mutant p53 (*p* value = 0.013) Fig. 5 (G).

Expression levels of genes PNPLA2 and PNPLA7 were found to be high in BC patients who expressed hormone estrogen and progesterone receptors (*p* value < 0.0001). In respect of SBR grading, increased levels of PNPLA2 and PNPLA7 were found to be connected with SBR1 (*p* value < 0.0001). Moreover, individuals who had wild p53 showed greater levels of PNPLA2 and PNPLA7 in comparison to those with mutant p53 Fig. 5H, I.

ACSL1 gene expression appeared to be elevated in breast tumors deficient in hormone estrogen and progesterone receptors (*p* value < 0.0001). In contrast, HER2-augmented breast tumors (*p* value-0.0001) showed elevated levels

A



B

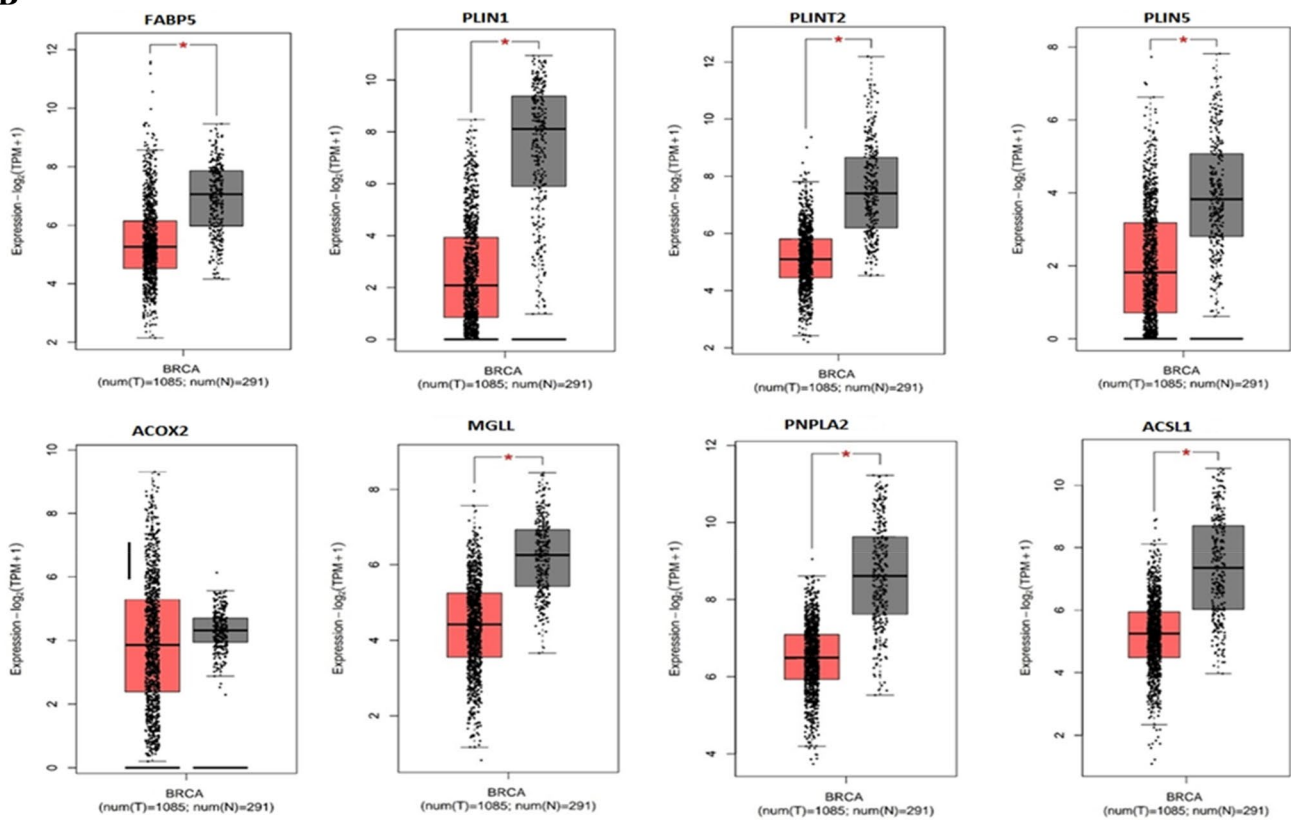


Fig. 2 Fatty acid metabolism gene expression in Breast cancer. **A.** Bar graph of the expression pattern of fatty acid metabolism genes scrutinized using TCGA BRCA and GTEx databases by operating

UCSC XENA. **B.** Box plots of the expression pattern of highly dys-regulated fatty acid metabolism genes analysed in BC by making use of Gepia2 web source (p value > 0.05)

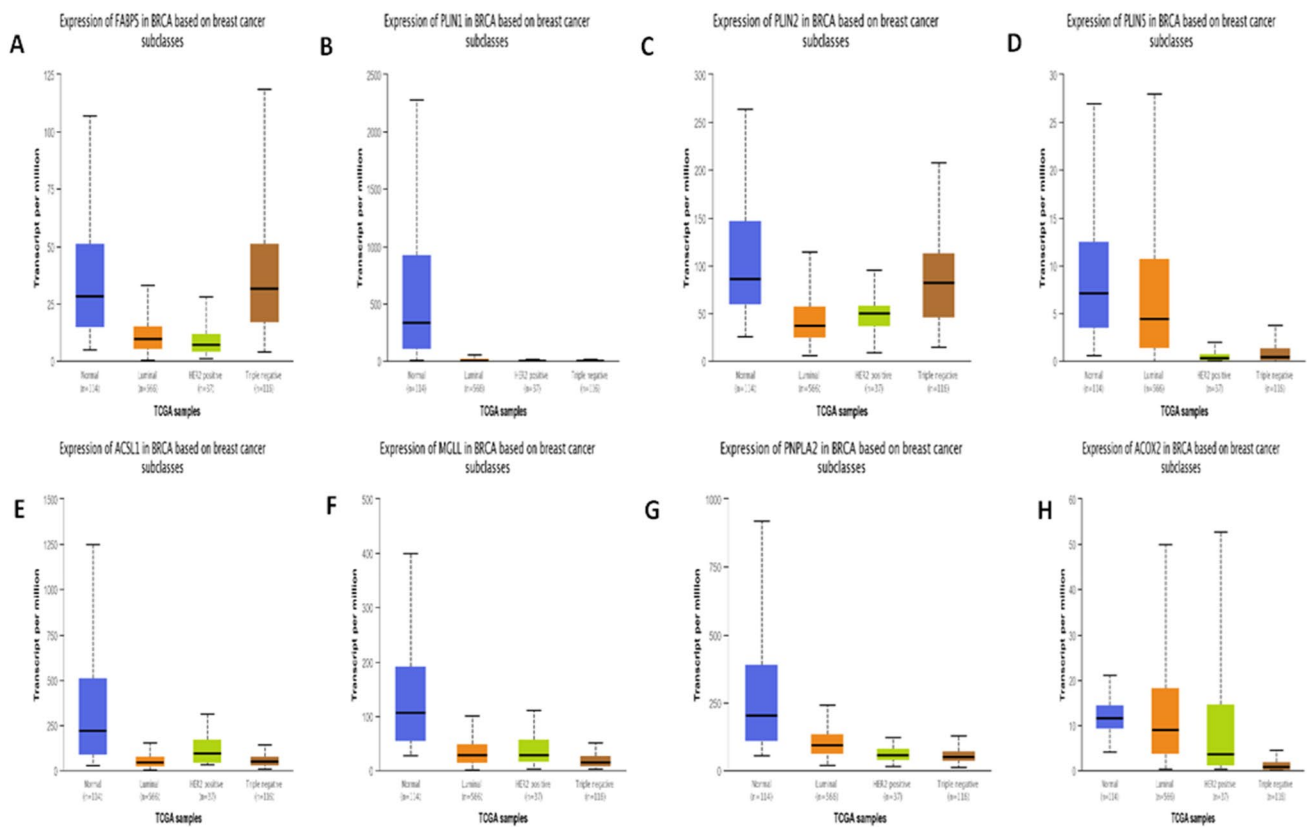


Fig. 3 Expression pattern of highly dysregulated Fatty acid metabolism genes in major BC subtypes. The expression analysis of the genes including **A** FABP5 **B** PLIN1 **C** PLIN2 **D** PLIN5 **E** ACSL1 **F** MGLL **G** PNPLA2 and **H** ACOX2 further explored using TCGA samples

of ACSL1. In respect of SBR grading, elevated ACSL1 expression was connected with SBR3 (p value < 0.0001). And individuals with mutant p53 had elevated levels of ACSL1 mRNA as opposed to those who had wild p53 (p value = 0.0517) Fig. 5J.

ACOX2 expression was high in patients with breast cancer who expressed progesterone and estrogen receptors (p value < 0.0001). ACOX2 expression was also found to be elevated in HER2-augmented breast tumors (p -value-0.0001). In respect of SBR grading, augmented ACOX2 expression was connected with SBR1 (p value < 0.0001), and individuals with wild p53 had remarkably higher levels of ACOX2 mRNA as opposed to patients with mutant p53 (p value < 0.0001) Fig. 5K.

Protein–protein interaction of Fatty Acid Metabolism Genes

A network of protein–protein interactions was constructed by connecting co-expressed 16 genes (nodes) with 62 protein–protein relationships (edges) in a string database. Also, the PPI network developed displayed an average node degree: 7.75, expected number of edges: 7, avg.

local clustering coefficient: 0.81, and a PPI enrichment p value: < 1.0e-16 (Fig. 6A). The top five hub genes of the network depending on degree score were identified using Cytohubba as shown in Fig. 6B. The top genes of the network included ACSL1, LIPE, PNPLA2, FABP4, and PPARG.

Gene ontology enrichment and pathways analysis

By utilizing the Enricher database, we pointed out signaling cascades and gene ontology features linked to FABP4, FABP5, PLIN1, PLIN2, PLIN4, PLIN5, LPIN1, MGLL, PNPLA2, PNPLA7, ACSL1, and ACOX2. GO analysis in biological processes revealed that these genes were enriched in long-chain fatty acid transport, regulation of sequestering of triglyceride fatty acid transport, acylglycerol catabolic process, triglyceride catabolic process, triglyceride metabolic process, acylglycerol acyl-chain remodeling, positive regulation of sequestering of triglyceride, positive regulation of cold-induced thermogenesis and regulation of triglyceride catabolic process Fig. 7A. In molecular function (MF) terms, these genes showed enrichment in activities of lipase, carboxylic ester hydrolase, phospholipase, lysophospholipase, arachidonate-CoA ligase, acid -thiol ligase, acylglycerol lipase, acyl-CoA oxidase, oxidoreductase, triglyceride

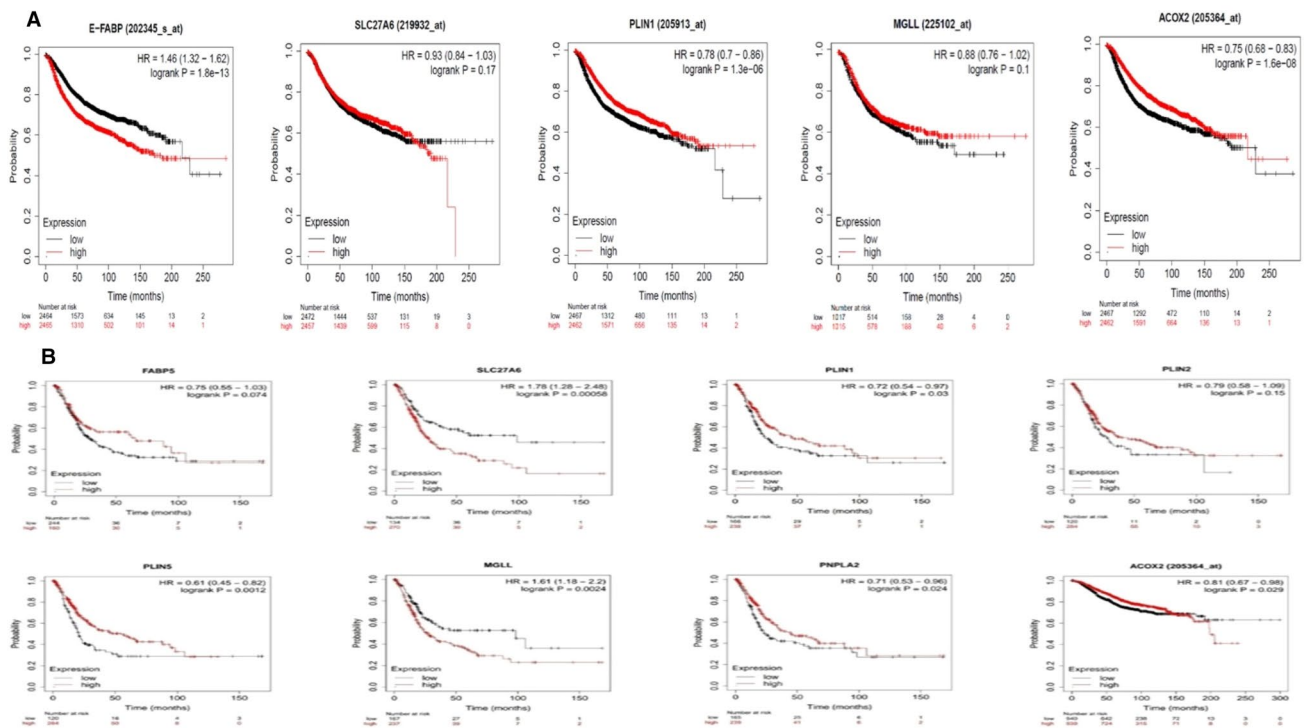


Fig. 4 Expression of dysregulated Fatty acid metabolism genes corresponds with survival outcomes. **A** Correspondence between expression of fatty acid metabolism genes with RFS in BC patients. **B** Relationship between expression of fatty acid metabolism genes with OS in BC patients

lipase, and long-chain fatty acid binding Fig. 7B. In respect of cellular location, the recommended GO was primarily enriched in lipid droplet, intracellular non-membrane abounded organelle, endoplasmic reticulum membrane, azurophil granule lumen, mitochondrial outer membrane, asymmetric synapse, postsynaptic density, organelle outer membrane, azurophil granule, vacuolar lumen, etc. Figure 7C.

Kyoto Encyclopaedia of Genes and Genomes (KEGG) web source was utilized to assess pathway studies. As per KEGG Human 2021, these genes are associated with the PPAR signalling pathway, regulation of lipolysis in adipocytes, thermogenesis, glycerolipid metabolism, peroxisome, primary bile acid biosynthesis, fatty acid biosynthesis, ferroptosis, fatty acid degradation, and adipocytokine signalling pathway Fig. 7D.

Discussion

Breast cancers are quite frequent among the female population and are responsible for increased tumor-related deaths in women [25], 34. While breakthroughs in early detection and therapy have improved the standard of life and overall survival in BC patients, the emergence of metastatic and resistant cancers demands immediate attention [26, 27].

Cancerous cells and their microenvironment including the stroma around them impact reciprocally via paracrine factors and juxtacrine interaction, promoting tumor development, metastasis, and therapy resistance [28]. The stromal cells have been discovered to generate epigenetic alterations in tumor cells and regulate gene expression, supporting tumor development and influencing prognosis [6, 29].

Several oncogenic signaling cascades regulate the lipid metabolism of transformed cells and any type of deviation in these pathways may lead to the development of tumors. Alterations in the metabolism of lipids in tumor cells are currently acknowledged as a trademark of transformed cells [30]. Reprogramming metabolic pathways by tumor cells is important for their proliferation and survival.

In the current study, we utilized various bioinformatic tools to puzzle out the diagnostic and prognostic significance, gene expression pattern, and functional role of the dysregulated fatty acid metabolism genes in BC patients. Our study reported that fatty acid metabolism genes are very much dysregulated in breast tumors with FABP4, FABP5, PLIN1, PLIN2, PLIN4, PLIN5, LPIN1, MGLL, PNPLA2, PNPLA7, ACSL1, and ACOX2 showing a fold change $> \pm 1.5$.

Our study revealed that breast cancer patients with low levels of PLIN1, MGLL, and ACOX2 have poor Relapse Free Survival as compared to patients having increased

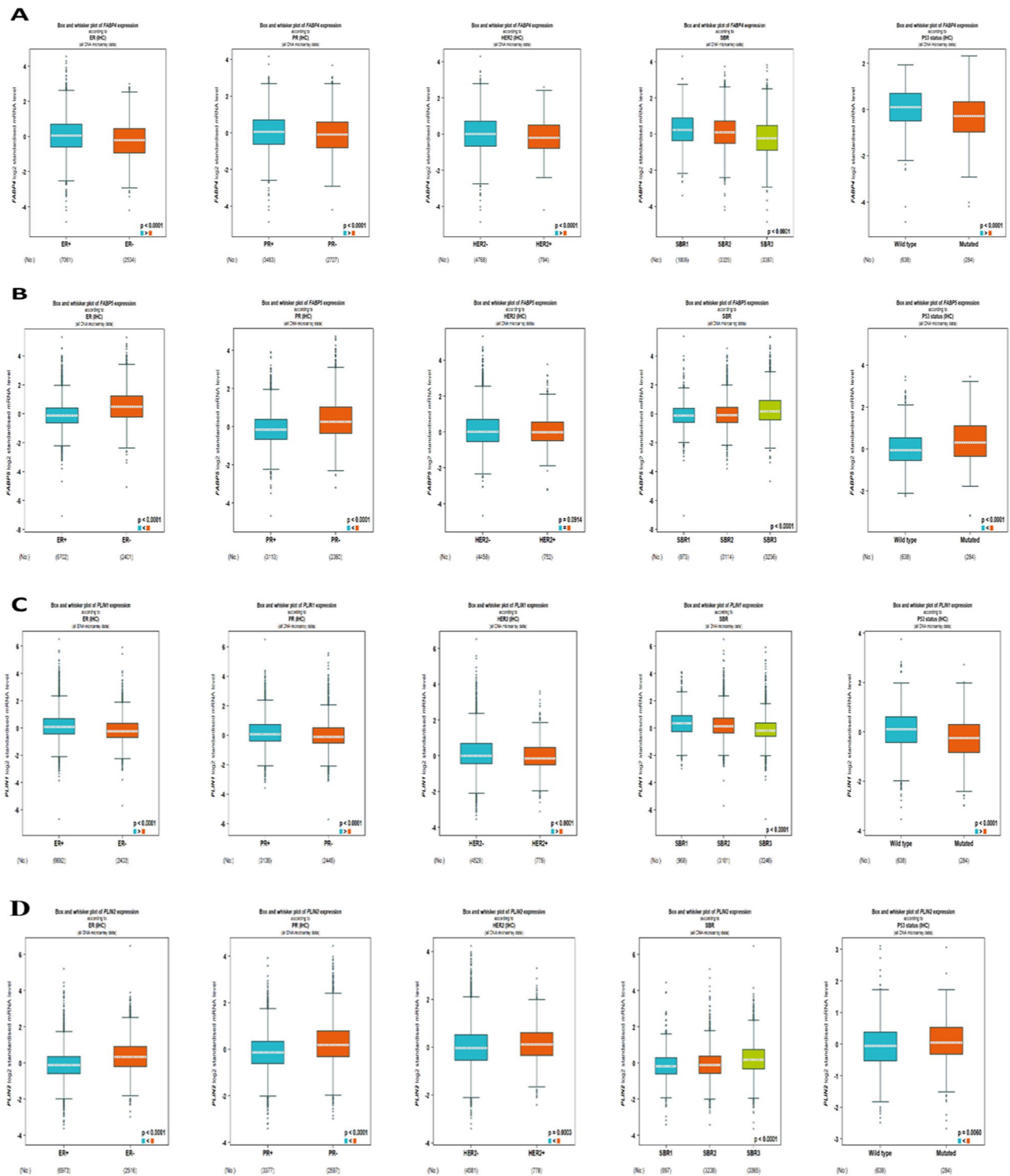


Fig. 5 Clinicopathological parameters and fatty acid metabolism genes in BC. bc-Gen EXminer analysis of dysregulated fatty acid metabolism genes showed a tie-up with clinicopathological param-

eters of BC i.e., Hormonal status, Her2 enrichment, SBR grade, and p53 status. **A** FABP4 **B** FABP5 **C** PLIN1 **D** PLIN2 **E** PLIN4 **F** PLIN5 **G** MGLL **H** PNPLA2 **I** PNPLA7 **J** ACSL1 **K** ACOX2

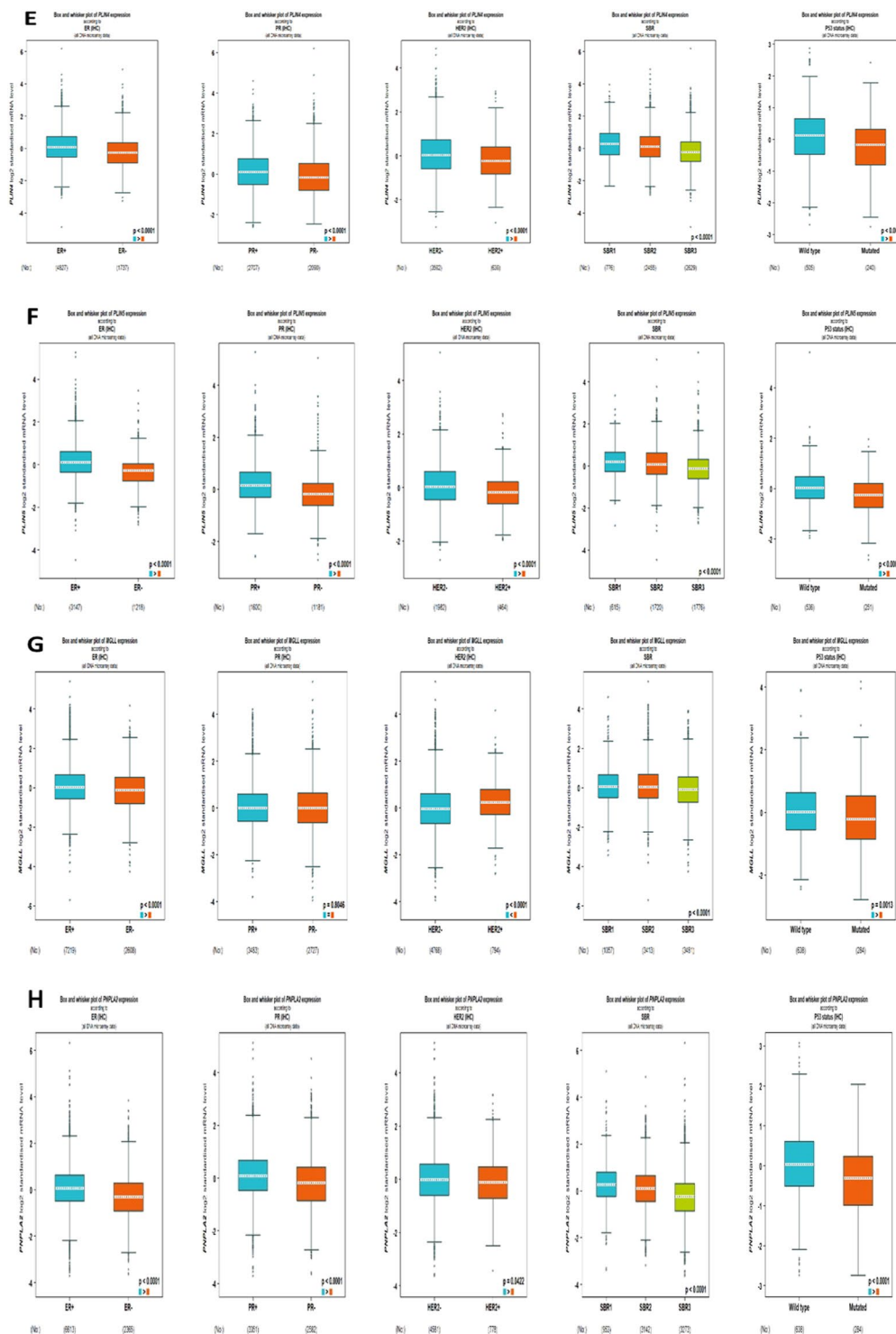


Fig. 5 (continued)

levels of PLIN1, MGLL, and ACOX2 mRNA, while patients having high expression of SLC27A6 and FABP5 show poor RFS. Further, it was reported that BC patients having elevated expression of SLC27A6, and MGLL, have poor

OS, while patients with low expression of FABP5, PLIN1, PLIN2, PLIN5 PNPLA2 and ACOX2 have worse OS.

Further investigation suggested that FABP4 expression was significantly higher in those BC patients who expressed hormone progesterone and estrogen receptors, depicting a

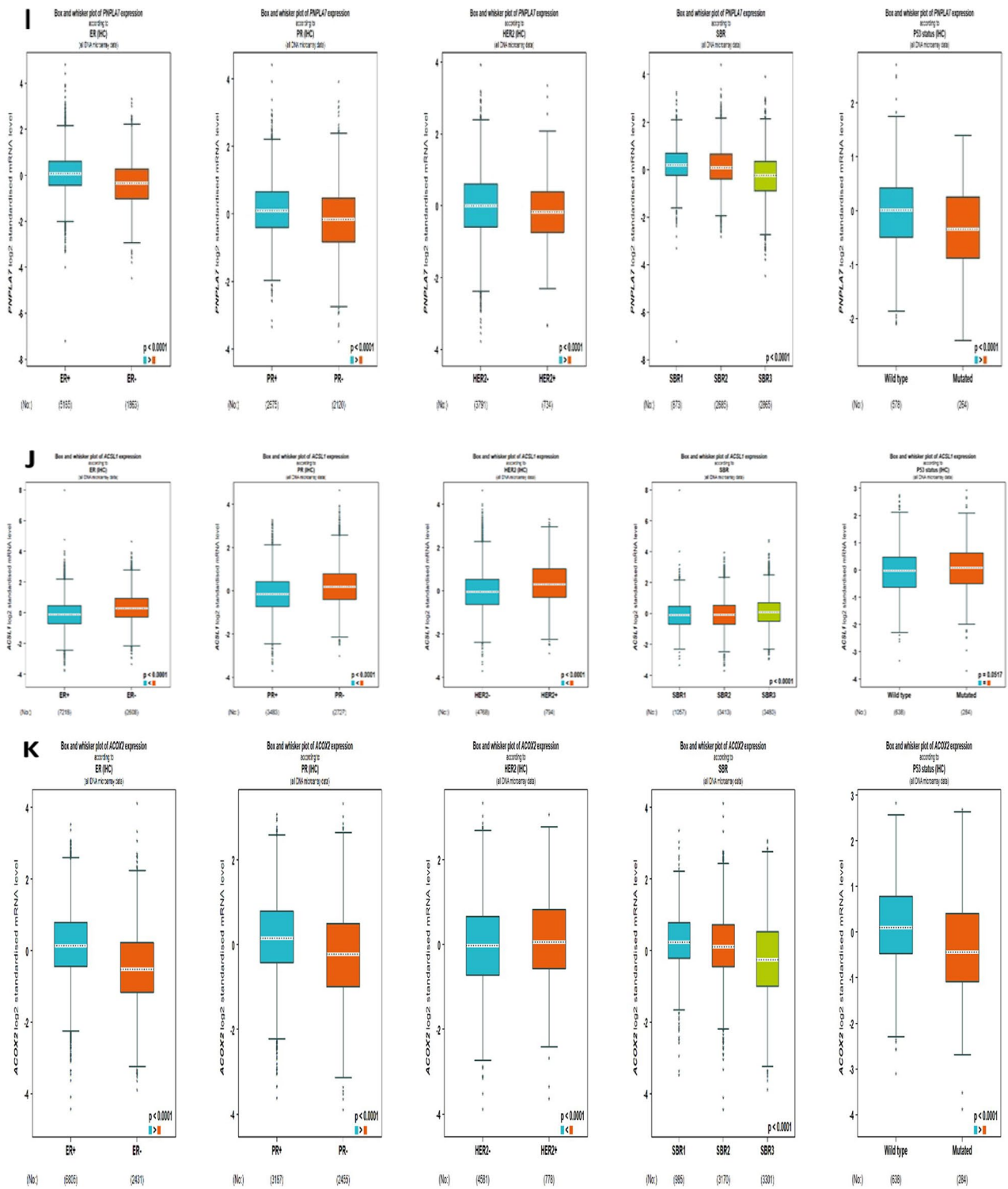


Fig. 5 (continued)

negative relationship with hormone receptors, and was observed to correlate with SBR grade 1 and wild p53. On the other hand, FABP5 expression appeared remarkably high in those patients deficient in estrogen and progesterone

receptors, however, did not vary substantially from HER2. In respect of SBR grading, elevated levels of FABP5 were found to be correlated with SBR3 and mutant p53.

Fig. 6 PPI analysis of FAM genes **A** PPI constricted module of FAM genes using STRING. **B** Top 5 hub genes of PPI network identified using cytohubba

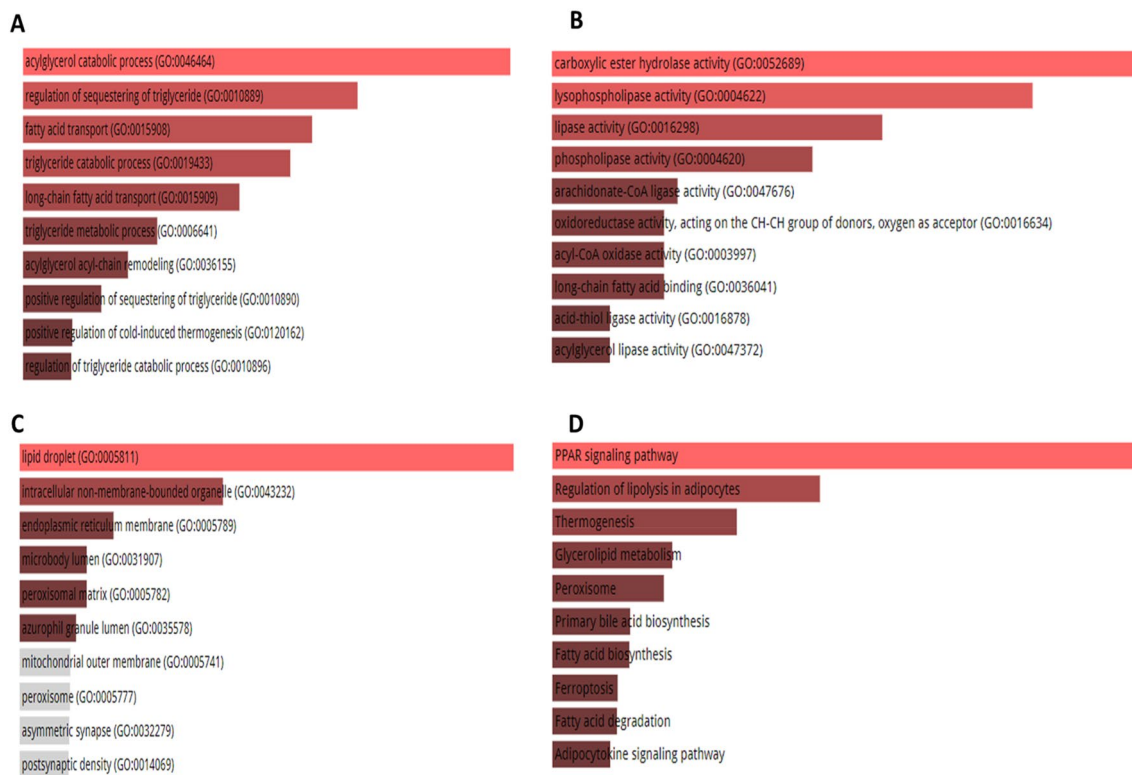
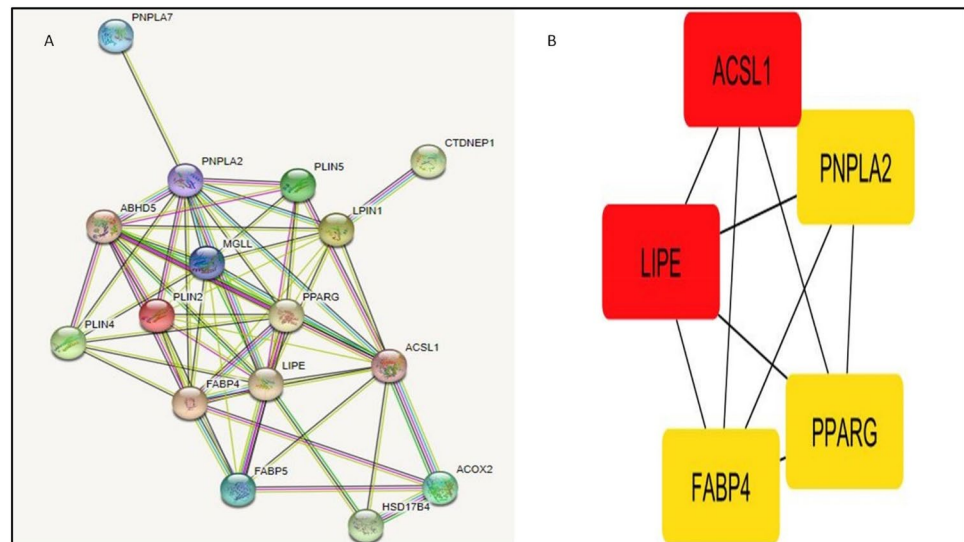


Fig. 7 Gene enrichment and pathway analysis. **(A–C)** GO analysis of the BP, CC, and MF and, **(D)** KEGG pathway enrichment analysis of FABP4, FABP5, PLIN1, PLIN2, PLIN4, PLIN5, LPIN1, MGLL, PNPLA2, PNPLA7, ACSL1 and ACOX2 genes in breast cancer

PLIN1 expression was high in BC patients who had estrogen receptors and progesterone receptors, depicting a negative relationship with hormone receptors and was associated with SBR1 and wild p53. Experimentally PLIN1 was overexpressed exogenously in breast cancer cell lines which resulted in the inhibition of invasion, cell proliferation, migration, and in vivo tumor formation in mice models

[31]. In contrast to this, expression levels of PLIN2 expression were enhanced in those patients not having estrogen and progesterone receptors and also enhanced in HER2-enriched breast cancers. In respect of SBR grading, elevated levels of PLIN2 appeared to be connected with SBR3 and mutant p53.

PLIN4 and PLIN5 expression was high in those patients having estrogen and progesterone receptors showing a negative relation with hormone receptors (p value < 0.0001). In respect of SBR grading, it was confirmed that elevated levels of PLIN4 and PLIN5 were connected with SBR1 and wild p53.

MGLL expression was high in ER + and HER2 positive patients while its expression did not vary very much from PR (p value = 0.8046) or SBR grade (p value < 0.0001) and individuals with wild-type p53 showed notably larger levels of MGLL mRNA as opposed to those with mutant p53.

PNPLA2 and PNPLA7 expression levels were elevated in BC patients having estrogen and progesterone receptors depicting a negative relation with hormone receptors. In respect of SBR grading, elevated levels of PNPLA2 & PNPLA7 appeared to be associated with SBR1 & wild p53.

Elevated expression of ACSL1 was reported in BC patients lacking progesterone and estrogen receptors, but its expression was augmented in HER2-enriched breast cancers. Concerning SBR grading, higher ACSL1 expression was found to be associated with SBR3 and mutant p53.

BC patients with estrogen and progesterone receptors exhibited elevated levels of ACOX2 depicting a positive relation. Its expression was also enhanced in HER2-enriched breast cancers. About SBR grading, elevated ACOX2 expression was connected with SBR1 & wild p53.

Also, the string analysis using the Cytoscape as export software revealed the top five hub genes associated with fatty acid metabolism in breast cancer. Further, Gene ontology analysis in biological processes revealed that these genes were enriched in long-chain fatty acid transport, acylglycerol catabolic process, triglyceride metabolic process, regulation of sequestering of triglyceride fatty acid transport, triglyceride catabolic process, acylglycerol acyl-chain remodeling, positive regulation of cold-induced thermogenesis, positive regulation of sequestering of triglyceride and regulation of triglyceride catabolic process. In molecular function (MF) terms, these genes showed enrichment in long-chain fatty acid binding and activities of carboxylic ester hydrolase, lysophospholipase, lipase, phospholipase, arachidonate -CoA ligase, acylglycerol lipase, acid-thiol ligase, CoA- ligase, triglyceride lipase, etc. About cellular location, the preferred GO was primarily enriched in lipid droplet, intracellular non-membrane abounded organelle, endoplasmic reticulum membrane, azurophil granule lumen, mitochondrial outer membrane, asymmetric synapse, post-synaptic density, organelle outer membrane, azurophil granule, vacuolar lumen, etc.

This dependency of transformed cells on the deviated metabolism of lipids demonstrates that proteins that function in lipid metabolism may be pinpointed as chemotherapeutic targets for the treatment of breast cancer [32, 33]

Materials and methods

Differential gene expression of fatty acid metabolism genes in breast cancers

The expression pattern of fatty acid metabolism genes was analyzed using the UCSC XENA web source and resulted in the production of a heatmap [35]. The Gepia2 online dataset (<http://gepia2.cancer-pku.cn/>) was utilized to show the fold change in differential expression levels of fatty acid metabolism genes in breast tumors [36]. This web source has 291 samples of GTEX & 1085 samples of BC. Additionally, our study also used UALCAN comprehensive web portal just to delineate the expression of highly dysregulated fatty acid metabolism genes in different subclasses of breast cancers [37].

Kaplan–Meier plotter

The Kaplan–Meier plotter, a bioinformatic tool for gene expression pattern information and survival probability of BC patients, was used to look over the survival time of highly dysregulated fatty acid metabolism genes [38]. This web server has OS data for 1879 patients and RFS data for 4929 patients in its mRNA gene chip BC dataset. Two groups of patients were created based on median gene expression. Among these two designated cohorts, consequences of dysregulated gene expression on OS and RFS were inspected from KM- survival curves. Moreover, the KM plotter was used to calculate log-rank p value and the hazard ratio intervals.

bc-Gen EXminer

The bc-Gen EXminer web source (<http://bcgenex.centre-gauducheau.fr/BC-GEM>) was used to analyze the correlation between highly dysregulated fatty acid metabolism genes and ordinary pathophysiological characteristics in BC patients [39, 40]. This database was utilized to assess the correlation between expression levels of different fatty acid metabolism genes and different clinical parameters of breast tumor patients such as Her2 enrichment, p53 status, hormonal status, and SBR grade.

Protein–protein interaction of Fatty acid metabolism genes

A protein–protein interaction (PPI) network of selected FAM genes was created using the Search Tool for the Retrieval of Interacting Genes (STRING database ver.11.0b), a biological database created to construct and study functional interactions between proteins, with a confidence score of

0.7 [41]. Using the Cytoscape program, the PPI network was further examined and visualized (version 3.8.2). To identify the noteworthy modules in the PPI network, the Cytoscape software's Molecular Complex Detection (MCODE) plugin was added. The cytohubba plugin was used to extract the HUB nodes with the top 5° in the PPI network [42].

Functional enrichment of highly dysregulated fatty acid metabolism genes

The Mayaan laboratory database (<https://maayanlab.cloud/Enrichr/>) meant for gene ontology function and KEGG (Kyoto Encyclopaedia of Genes and Genomes) was utilized to reveal biological process, gene ontology features, and pathway enrichment analyses of FABP4, FABP5, PLIN1, PLIN2, PLIN4, PLIN5, LPIN1, MGLL, PNPLA2, PNPLA7, ACSL1 and ACOX2 [43]. A *p* value of < 0.05 was considered significant for pathways & functions in respect of KEGG & GO.

Conclusion

In this study, a correlation was assessed between expression levels of different fatty acid metabolism genes and prognosis among different molecular isotypes/subtypes of breast cancers. Our findings suggest that FAM genes viz, FABP4, FABP5, PLIN1, PLIN2, PLIN4, PLIN5, LPIN1, MGLL, PNPLA2, PNPLA7, ACSL1, and ACOX2 are downregulated in different subtypes of breast cancers, and may serve as ideal therapeutic targets for cancer treatment. The studies have revealed that PLIN1 overexpression done exogenously in breast cancer cell lines resulted in inhibition of proliferation, migration, invasion as well as in vivo tumor formation in mice models, hence normal activation of fatty acid metabolism pathways in tumors may reduce abnormal cell proliferation and may give better treatment outcomes. Therefore, targeting the fatty acid metabolism pathway and the genes related to it will prove an efficient treatment strategy for breast cancer patients.

Acknowledgements Mr Umar Yousuf Bhat is a recipient of UGC CSIR JRF, Govt of India. The authors would like to thank Mr. Umar Mehraj for helping Miss Aanisa Makhdoomi (Masters student) during her project work.

Author contributions MAM: conceptualization, funding acquisition, supervision, review, and editing. MAM and UY collected the data and performed the bioinformatic analysis. UY and SS wrote the manuscript. UY and AA designed the figures. MAM edited the manuscript. All the authors critically revised read and approved the final manuscript.

Funding The work was supported by a research grant sanctioned to Manzoor Ahmad Mir by Jammu and Kashmir Science Technology and

Innovation Council (JKST&IC), Department of science and technology, Govt of J&K vide Grant Number. JKST&IC/SRE/885–87.

Declarations

Conflict of interest The authors declare that they have no conflict (s) of interest in this research.

References

- Anastasiadi Z, et al. Breast cancer in young women: an overview. *Updat Surg*. 2017;69(3):313–7.
- Mehraj U, et al. Chemokines in triple-negative breast cancer heterogeneity new challenges for clinical implications. *Semin Cancer Biol*. 2022. <https://doi.org/10.1016/j.semcancer.2022.03.008>.
- Mehraj U, et al. Expression pattern and prognostic significance of CDKs in breast cancer: an integrated bioinformatic study. *Cancer Biomark*. 2022;34(3):505–19.
- Sofi S, et al. Cyclin-dependent kinases in breast cancer: expression pattern and therapeutic implications. *Med Oncol*. 2022;39(6):1–16.
- Palmer JR, et al. A validated risk prediction model for breast cancer in US Black Women. *J Clin Oncol*. 2021;39(34):3866–77.
- Mehraj U, et al. Cryptolepine targets TOP2A and inhibits tumor cell proliferation in breast cancer cells-an in vitro and in silico study. *Anticancer Agents Med Chem*. 2022;22(17):3025–37.
- Brigham, et al. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012;490(7418):61–70.
- Mehraj U, et al. Expression pattern and prognostic significance of baculoviral inhibitor of apoptosis repeat-containing 5 (BIRC5) in breast cancer: a comprehensive analysis. *Adv Cancer Biol-Metastas*. 2022;4:100037. <https://doi.org/10.1016/j.adcanc.2022.100037>.
- Bhargava R, et al. Prevalence, morphologic features and proliferation indices of breast carcinoma molecular classes using immunohistochemical surrogate markers. *Int J Clin Exp Pathol*. 2009;2(5):444.
- Al Tamimi DM, et al. Protein expression profile and prevalence pattern of the molecular classes of breast cancer-a Saudi population based study. *BMC Cancer*. 2010;10(1):1–13.
- Mehraj U, Qayoom H, Mir MA. Prognostic significance and targeting tumor-associated macrophages in cancer: new insights and future perspectives. *Breast Cancer*. 2021;28(3):539–55.
- Mehraj U, et al. Expression pattern and prognostic significance of chemokines in breast cancer: an Integrated Bioinformatics Analysis. *Clin Breast Cancer*. 2022;22(6):567–78. <https://doi.org/10.1016/j.clbc.2022.04.008>.
- Fromer M. New SEER report documents high risk of second cancers in cancer survivors. *Oncol Times*. 2007;29(5):8.
- Yin L, et al. Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Res*. 2020;22(1):1–13.
- Mehraj U, et al. Adapalene synergistically with doxorubicin promotes apoptosis of TNBC Cells by hyperactivation of the ERK1/2 pathway through ROS induction. *Front Oncol*. 2022;12:1–15. <https://doi.org/10.3389/fonc.2022.938052>.
- Gluz O, et al. Triple-negative breast cancer—current status and future directions. *Ann Oncol*. 2009;20(12):1913–27.
- Qayoom H, et al. An insight into the cancer stem cell survival pathways involved in chemoresistance in triple-negative breast cancer. *Future Oncol*. 2021;17(31):4185–206.
- Barba D, et al. Breast cancer, screening and diagnostic tools: all you need to know. *Crit Rev Oncol Hematol*. 2021;157:103174.

19. Dent R, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res*. 2007;13(15):4429–34.
20. Mir, MA., *Combinational Therapy in Triple Negative Breast Cancer - 1st Edition*. 2021. ISBN: 9780323961363, Elsevier Inc. Publishers USA, 2022. 1(1): 1–242. <https://doi.org/10.1016/C2021-0-02565-7>
21. Mir, M., *Combination Therapies and their Effectiveness in Breast Cancer Treatment*. 2021. ISBN: 978-1-68507–195–0, Nova Biomedical Science Publishers USA, 2021.1(1): p. 1–411. <https://doi.org/10.52305/WXJL6770>
22. Mir, M.A., *Developing costimulatory molecules for immunotherapy of diseases*. 2015. 1(1): p.1–299. Academic Press Elsevier USA. ISBN: 9780128025857, <https://doi.org/10.1016/C2014-0-02898-5>
23. Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat Rev Cancer*. 2007;7(10):763–77.
24. Puig T, et al. Fatty acid metabolism in breast cancer cells: differential inhibitory effects of epigallocatechin gallate (EGCG) and C75. *Breast Cancer Res Treat*. 2008;109(3):471–9.
25. Monaco ME. Fatty acid metabolism in breast cancer subtypes. *Oncotarget*. 2017;8(17):29487.
26. Hu Z, et al. The molecular portraits of breast tumors are conserved across microarray platforms. *BMC Genomics*. 2006;7(1):1–12.
27. Qayoom H, Bhat BA, Mehraj UU, Mir MA. Rising trends of cancers in Kashmir valley: distribution pattern, incidence and causes. *J Oncol Res Treat*. 2020;5(2):1–13.
28. Mehraj U, et al. The tumor microenvironment as driver of stemness and therapeutic resistance in breast cancer: new challenges and therapeutic opportunities. *Cell Oncol*. 2021;44:1209–29. <https://doi.org/10.1007/s13402-021-00634-9>.
29. Mehraj U, et al. Tumor microenvironment promotes breast cancer chemoresistance. *Cancer Chemother Pharmacol*. 2021;87(2):147–58.
30. Mir MA, et al. Recent advances in metabolites from medicinal plants in cancer prevention and treatment. *Curr Immunol Rev*. 2019;15(2):185–201.
31. Tennant DA, Durán RV, Gottlieb E. Targeting metabolic transformation for cancer therapy. *Nat Rev Cancer*. 2010;10(4):267–77.
32. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer*. 2011;11(2):85–95.
33. Xu S, et al. Fatty acid synthase promotes breast cancer metastasis by mediating changes in fatty acid metabolism. *Oncol Lett*. 2021;21(1):1.
34. Mehraj U, et al. Adapalene inhibits the growth of triple-negative breast cancer cells by S-phase arrest and potentiates the antitumor efficacy of GDC-0941. *Front Pharmacol*. 2022;13: 958443. <https://doi.org/10.3389/fphar.2022.958443>.
35. Goldman MJ, et al. Visualizing and interpreting cancer genomics data via the Xena platform. *Nat Biotechnol*. 2020;38(6):675–8.
36. Tang Z, et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res*. 2017;45(W1):W98–102.
37. Chandrashekar DS, et al. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. *Neoplasia*. 2017;19(8):649–58.
38. Györfy B, et al. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast Cancer Res Treat*. 2010;123(3):725–31.
39. Jézéquel P, et al. bc-GenExMiner: an easy-to-use online platform for gene prognostic analyses in breast cancer. *Breast Cancer Res Treat*. 2012;131(3):765–75.
40. Jézéquel P, et al. (2013) bc-GenExMiner: 3.0 new mining module computes breast cancer gene expression correlation analyses. Database. 2013. <https://doi.org/10.1093/database/bas060>.
41. Szklarczyk D, et al. STRING v10: protein–protein interaction networks, integrated over the tree of life. *Nucleic Acids Res*. 2015;43(D1):D447–52.
42. Chin C-H, et al. cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst Biol*. 2014;8(4):1–7.
43. Kuleshov MV, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res*. 2016;44(W1):W90–7.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.