Received: 22 September 2020 | Revised: 6 November 2020 | Accepted: 10 November 2020

DOI: 10.1111/tbj.14119

ORIGINAL ARTICLE

The Breast Journal WILEY

Gene expression comparison between primary estrogen receptor-positive and triple-negative breast cancer with paired axillary lymph node metastasis

Marissa K. Srour MD[1] | Ying Qu PhD[2] | Nan Deng PhD[3] | Kjirsten Carlson MD[1] | James Mirocha MS[3] | Bowen Gao MS[2] | Farnaz Dadmanesh MD[4] | Xiaojiang Cui PhD[2] | Armando E. Giuliano MD[1]

[1]Department of Surgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA

[2]Department of Surgery, Cedars-Sinai Medical Center, Samuel Oschin Comprehensive Cancer Institute, Los Angeles, CA, USA

[3]Biostatistics and Bioinformatics Research Center, Cedars-Sinai Medical Center, Samuel Oschin Comprehensive Cancer Institute, Los Angeles, CA, USA

[4]Department of Pathology, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Correspondence
Armando E. Giuliano, MD, Department of Surgery – Division of Surgical Oncology, Cedars-Sinai Medical Center, 310 North San Vicente Blvd, Los Angeles, CA 90048, USA.
Email: armando.giuliano@cshs.org

Funding information
Margie and Robert E. Petersen Foundation; U.S. Department of Defense, Grant/Award Number: W81XWH-18-1-0067; Samuel Oschin Cancer Institute Discovery Fund Award; Community Outreach and Engagement Developmental Fund Award; Fashion Footwear Association of New York; NIH Clinical Center, Grant/Award Number: 2R01CA151610; Linda and Jim Lippman

Abstract

The aim of this study is to characterize and compare changes in gene expression patterns of paired axillary lymph node (ALN) metastases from estrogen receptor (ER)-positive and triple-negative (TNBC) primary breast cancer (PBC). Patients with stage 2-3 PBC with macrometastasis to an ALN were selected. Gene expression of 2567 cancer-associated genes was analyzed with the HTG EdgeSeq system coupled with the Illumina Next Generation Sequencing (NGS) platform. Changes in gene expression between ER/PR-positive, HER2-negative PBC, and their paired ALN metastases were compared with TNBC and their paired ALN metastases. Fourteen pairs of ER-positive and paired ALN metastasis were analyzed. Compared with the PBC, ALN metastasis had 673 significant differentially expressed genes, including 348 upregulated genes and 325 downregulated genes. Seventeen pairs of TNBC and paired ALN metastasis were analyzed. ALN metastasis had 257 significant differentially expressed genes, including 123 upregulated genes and 134 downregulated genes. When gene expression of the ALN for ER-positive PBC was compared to that of TNBC, 97 genes were upregulated in both, and 115 genes were similarly downregulated. Common upregulated genes were associated with cell death, necrosis, and homeostasis. Common downregulated genes were those of migration, degradation of extracellular matrix, and invasion. Although ER-positive PBC and TNBC have a distinct gene expression profiles and distinct changes from PBC to ALN metastases, a significant number of genes are similarly up- or downregulated. Understanding the role of these common genomic changes may provide clues to understanding the metastatic process itself.

KEYWORDS

estrogen receptor-positive breast cancer, gene expression, lymph node metastasis, triple-negative breast cancer

Meeting Presentation: Presented at the annual meeting of the Society of Surgical Oncology (SSO), virtual podium presentation, August 2020.

1 | INTRODUCTION

Axillary lymph node (ALN) metastases often guide breast cancer treatment and prognosis, but are early manifestations of cancer spread. Understanding the genomics of this early spread may provide clues to understanding the metastatic process itself. While estrogen receptor (ER)-positive breast cancer comprises the majority of all breast cancer, triple-negative breast cancer (TNBC) is an important subtype that comprises 10%-15% of all breast cancer. Similar rates of nodal metastasis occur between these two very different breast cancer subtypes.[1] Understanding lymph node metastasis could aid not only in understanding breast cancer biology, but also in developing new therapies.[2-6]

To date, there have been few studies comparing the genomics of primary breast cancer (PBC) of different subtypes with their axillary metastases. Prior research evaluating gene expression of primary breast cancer (PBC) with paired lymph node metastases had inconsistent outcomes and rarely evaluated patients with TNBC. For the studies that did include TNBC, pooled data were used, and biomarker profile was not controlled for.

The objective of this study was to characterize and compare changes in gene expression patterns of paired ALN metastases from ER-positive and triple-negative PBC and to identify common genomic patterns that may provide clues to understanding the metastatic process.

2 | METHODS

2.1 | Patient selection

After Institutional Review Board (IRB) approval, a prospectively maintained database was searched from January 2007 to December 2016 to identify patients with stage 2-3 invasive ductal carcinoma (IDC) with lymph node macrometastasis. According to the American Society of Clinical Oncology (ASCO) and College of American Pathologists guidelines, tumors that were estrogen receptor-positive, progesterone receptor-positive, and HER2-neu-negative were classified as "ER positive." Tumors that were estrogen receptor-negative, progesterone receptor-negative, and HER2-neu-negative were classified as "triple negative" and were further selected for inclusion in the study.[7] ER and PR expression was determined by immunohistochemistry with tumors expressing ER in ≥1% of tumor cells or PR in ≥1% of tumor cells, respectively.

As previously described in our methods from our institution on gene expression,[8] the sentinel lymph node was selected for the study if a sentinel node biopsy was performed and it harbored metastasis. When an axillary lymph node dissection was performed, the lymph node with the largest macrometastasis was selected for the study. Additional patient inclusion criteria included primary breast cancer tumor size <5 cm and lymph node metastasis tumor size >5 mm. Patients were excluded if they received neoadjuvant chemotherapy or hormonal therapy, had distant metastatic disease at diagnosis, or a personal history of a previous primary breast cancer.

2.2 | Tissue samples

Formalin-fixed paraffin-embedded (FFPE) tissue blocks of the primary breast cancer (PBC) and paired (from the same patient) lymph node metastasis were selected. Unstained FFPE tissue sections of the PBC and lymph node metastasis were cut at 4 μm thickness and mounted onto glass slides. Using visualization under the microscope, the tumor area was marked by a pathologist on a corresponding hematoxylin and eosin (H&E) stained section for both the PBC and the paired lymph node metastasis. The tissue was submitted to HTG for further processing, where the tissue was removed from the marked section and placed into the HTG lysis buffer (see methods below).[8]

2.3 | HTG EdgeSeq combined with next generation sequencing

As previously reported,[8] HTG EdgeSeq system combines HTG's proprietary quantitative nuclease protection assay (qNPA) chemistry with Illumina Next Generation Sequencing (NGS) platform to enable a semi-quantitative analysis of a panel of targeted genes in a single assay. This allows for mRNA quantization without nucleic acid extraction. Samples are added to the HTG lysis buffer. Functional DNA nuclease protection probes (NPPs) flanked by universal sequences were hybridized to target RNAs. Universal DNA wingmen probes were hybridized to the wings to prevent S1 nuclease digestion. Next, S1 nuclease was added to digest excess nonhybridized DNA probes and nonhybridized RNA. Heat denaturation was used to release the protection probes from the DNA:RNA duplexes. Target capture was performed with polymerase chain reaction (PCR) amplification and barcoding. Sequencing was performed using Illumina NextSeq platform. All samples passed HTG postsequencing metrics.[9]

2.4 | HTG EdgeSeq oncology biomarker panel

An HTG EdgeSeq oncology biomarker panel which is an assay containing probes for 2567 genes, including 15 housekeeper genes, four negative process controls, and four positive process controls was selected for use on each PBC and lymph node metastasis tumor.[10]

2.5 | Data analysis

As described,[8] data were returned from the sequencer as a demultiplexed FASTQ file, with four files per original assay well. The relative expression abundances of genes were calculated as count per million (CPM) within each sample. This result allows for evaluation of gene expression as a proportion of total counts on a sample level.

TABLE 1 Clinical and tumor characteristics

	All patients	ER-positive	TNBC	
	(N = 31)	(N = 14)	(N = 17)	*P*-value
Age (y), median, (range)	62 (30-88)	61 (35-79)	62 (30-88)	.72
Type of operation				
Partial mastectomy	26 (83.9)	11 (78.6)	15 (88.2)	.64
Mastectomy	5 (16.1)	3 (21.4)	2 (11.8)	
Histology				
IDC	31 (100.0)	14 (100.0)	17 (100.0)	—
Subtype				
ER-positive	14 (45.2)	14 (100.0)	0 (0.0)	—
TNBC	17 (54.8)	0 (0.0)	17 (100.0)	—
Grade				
Well differentiated	1 (3.2)	1 (7.1)	0 (0.0)	.01
Moderately differentiated	14 (45.2)	10 (71.4)	4 (23.5)	
Poorly differentiated	16 (51.6)	3 (21.4)	13 (76.5)	
Tumor size (cm), median (range)	2.4 (1.2-4.5)	2.2 (1.2-4.5)	2.5 (1.2-4.0)	.06
Focality				
Single focus	17 (54.8)	11 (78.6)	16 (94.1)	.31
Multifocal	4 (12.9)	3 (21.4)	1 (5.9)	
LVI	19 (61.3)	6 (42.9)	13 (76.5)	.08
DCIS	28 (90.3)	13 (92.9)	15 (88.2)	.99
Size DCIS (cm), median (range)	1.4 (0.1-4.0)	1.2 (0.3-3.0)	2.5 (0.1-4.0)	.59
Ki-67 (%), median (range)	15 (1-86)	15 (2-53)	70 (1-86)	.007
Low (<11%)	8 (25.8)	6 (42.9)	2 (11.8)	.043
Intermediate (11%-20%)	6 19.4)	3 (21.4)	3 (17.7)	
High (>20%)	17 (54.8)	5 (35.7)	12 (70.6)	
Genetic mutation				
BRCA1	3 (9.7)	0 (0.0)	3 (17.6)	.23
BARD1	1 (3.2)	0 (0.0)	1 (5.9)	
Pathologic T stage				
1	8 (25.8)	5 (35.7)	3 (17.6)	.41
2	23 (74.2)	9 (64.3)	14 (82.4)	
Pathologic N stage				
1	26 (83.9)	13 (92.9)	13 (76.5)	.61
2	4 (12.9)	1 (7.1)	3 (17.7)	
3	1 (3.2)	0 (0.0)	1 (5.9)	
Stage				
2	26 (83.9)	13 (92.9)	13 (76.5)	.34
3	5 (16.2)	1 (7.1)	4 (23.5)	

Abbreviations: ALND, axillary lymph node dissection; DCIS, ductal carcinoma in situ; ER, estrogen receptor; IDC, intraductal carcinoma; LN, lymph node; LVI, lymphovascular invasion; SNB, sentinel lymph node biopsy; TNBC, triple-negative breast cancer.

Fifteen genes with average abundance <5 CPM were filtered out. Read counts of remaining 2544 genes were used to calculate scaling factors with trimmed mean of M-value (TMM) method for normalization. The differential expression (DE) analysis was performed by fitting a negative binomial generalized log-linear model for each gene using the edgeR package (v 3.24.3).[10] For the comparison between ALN and PBC, the DE analysis was adjusted by patients. The p-values of multiple tests were adjusted using Benjamini-Hochberg method,[11] and the significant level is designed as false discovery rate (FDR) <0.05.

2.6 | Statistical analysis

The upregulation and downregulation of genes were reviewed and the fold change (FC) was defined as the ratio of the normalized intensity of axillary lymph node metastasis to normalized intensity in the PBC. A FC >1 meant genes were upregulated in the ALN compared to the PBC. A FC between 0 and 1 meant genes were downregulated in the ALN compared to the PBC. Genes expression was considered significantly differentially expressed if they had an adjusted *P*-value, FDR <.05. The functions of the differentially expressed genes were retrieved using National Center for Biotechnology Information (NCBI) genomic data source.

For Tables 1 and 2, numerical variables were summarized by median and range, and compared across groups by the Wilcoxon rank sum test (exact version). Categorical variables were summarized by frequency and percentage and were compared across groups by the Fisher exact test. A two-sided 0.005 significance was used throughout. SAS version 9.4 (SAS Institute) was used for statistical calculations.

2.7 | Comparison

Changes in gene expression between the 14 pairs of ER-positive PBC and their paired ALN metastases were compared with the 17 pairs of TNBC and their paired ALN metastases. For common upregulated and downregulated genes, the fold change is reported for the TNBC compared to the ER-positive PBC.

2.8 | Reactome analysis and canonical pathways

Reactome is a curated database of pathways and reactions in human biology, and defines a "reaction" as any event in biology that changes the state of a biologic molecule.[12] Binding, activation, translocation, degradation, and classical biochemical events involving a catalyst are all reactions. Reactome's annotated data describe reactions possible if all annotated proteins and small molecules were present and active simultaneously in a cell. By overlying an experimental dataset on these annotations, a pathway analysis is performed. A binomial test is used to calculate the probability shown for each result, and the *P*-values are corrected for the multiple testing (Benjamani-Hochberg procedure) that arises from evaluating the submitted list of identifiers against every pathway. A reactome analysis for common genes of ER-positive and TNBC was performed. For canonical pathways, the "Common Genes" analysis predicts the downstream biologic processes, which are increased or decreased based on input data.[13] All analyses were carried out with differentially expressed genes with an FDR <0.05.

3 | RESULTS

3.1 | Clinicopathologic characteristics

When comparing women with ER-positive to those with TNBC, there was no difference between the groups in age (61 years ER-positive vs. 62 years TNBC, $P = .72$) or median PBC tumor size

TABLE 2 Lymph node characteristics

	All patients (N = 31)	ER-positive (N = 14)	TNBC (N = 17)	*P*-value
Axillary operation				
SNB	13 (41.9)	7 (50.0)	6 (35.3)	.046
ALND	9 (29.0)	1 (7.1)	8 (47.1)	
SNB + ALND	9 (29.0)	6 (42.9)	3 (17.6)	
Axillary lymph node metastasis	31 (100.0)	14 (100.0)	17 (100.0)	—
Number LN sampled, average (range)	7 (1-20)	4 (1-17)	13 (1-20)	.01
Number positive LN, median (range)	2 (1-7)	2 (1-7)	2 (1-4)	.07
LN tumor size (cm), median (range)	0.9 (0.5-2.5)	0.8 (0.5-1.1)	1.0 (0.6-2.5)	.11
Extranodal extension	17 (54.8)	9 (64.3)	8 (47.1)	.47
Size extranodal extension (cm), median (range)	0.2 (0.1-0.5)	0.2 (0.1-0.5)	0.4 (0.25-0.6)	.02
SLN used	16 (51.6)	8 (57.1)	8 (47.1)	.72

Abbreviations: ALND, axillary lymph node dissection; DCIS, ductal carcinoma in situ; IDC, intraductal carcinoma; LN, lymph node; LVI, lymphovascular invasion; SNB, sentinel lymph node biopsy; TNBC, triple-negative breast cancer.

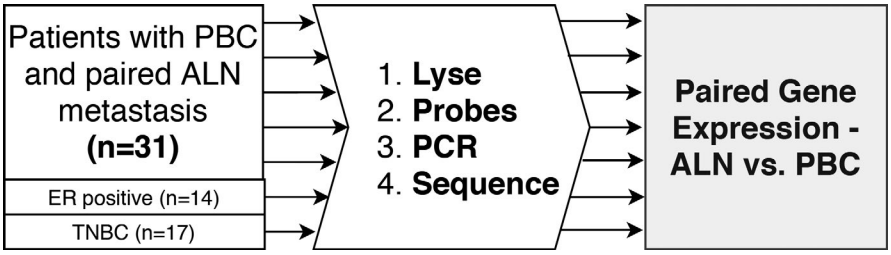

FIGURE 1 Methods for paired gene expression analysis. ALN, axillary lymph node; ER, estrogen receptor; PBC, primary breast cancer; PCR, polymerase chain reaction; TNBC, triple negative breast cancer

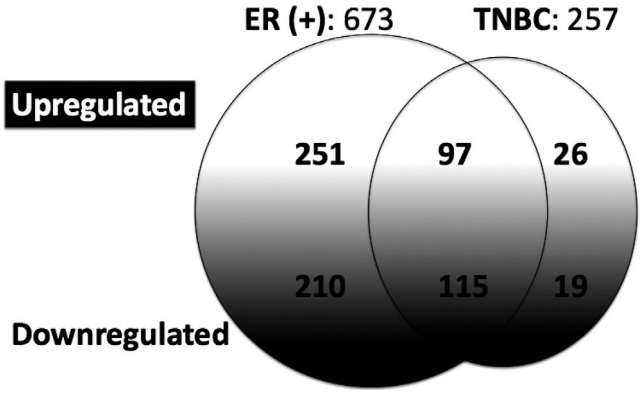

FIGURE 2 Venn Diagram of the 718 Differentially Expressed Genes Estrogen Receptor (ER)-positive and Triple-Negative Breast Cancer (TNBC). A white color gradient (top) represents the upregulated genes, and a black color gradient (bottom) represents the downregulated genes. The circles of the diagram represent ER-positive (left) and TNBC (right). There were 718 differentially expressed genes whose expression was statistically significant. From that group, 97 genes were upregulated in both tumor types (top middle), and 115 genes were similarly downregulated (bottom middle). In ER-positive primary breast cancer, axillary lymph node (ALN) metastasis had 251 uniquely upregulated genes (top left), and 210 uniquely downregulated genes (bottom left). In TNBC, ALN metastasis had 26 genes uniquely upregulated genes (top right), and 19 uniquely downregulated genes (bottom right)

(2.2 cm ER-positive vs. 2.5 cm TNBC, $P = .06$). The majority of patients had stage 2 disease (92.9% ER-positive vs. 76.5% TNBC, $P = .34$). Additional clinical and pathologic characteristics are shown in Table 1. Median lymph node tumor size was 0.9 cm (0.8 cm ER-positive vs. 1.0cm TNBC, $P = .11$). Sixteen (51.6%) patients had a SLN with metastasis examined, and 15 (48.4%) patients had a positive nonsentinel axillary node examined (Table 2).

3.2 | ALN compared to PBC—ER-positive

14 pairs of primary estrogen receptor-positive and paired ALN metastases were analyzed for 2567 cancer-associated genes (Figure 1). Compared with the PBC, ALN metastases had 673 statistically significant differentially expressed genes, including 348 upregulated genes and 325 downregulated genes (Figure 2). There were 60 upregulated genes that had $\log_2$FC ≥2.00 FC gene expression and 29 downregulated genes that had $\log_2$FC ≤−2.00 FC in gene expression, which is shown in Table 3.

3.3 | ALN compared to PBC—TNBC

Seventeen pairs of PBC and paired ALN metastases were analyzed for 2567 cancer-associated genes (Figure 1). As previously published, "Compared with the PBC, ALN metastases had 257 statistically significant differentially expressed genes, including 123 upregulated genes and 134 downregulated genes (Figure 2). There were 5 upregulated genes that had $\log_2$FC ≥2.00 FC gene expression and nine downregulated genes that had $\log_2$FC ≤−2.00 FC in gene expression, which is shown in Table 3."[8]

3.4 | Common upregulated genes: ALN compared to PBC

When gene expression of the ALN for ER-positive PBC was compared to that of TNBC, 97 genes were upregulated in both tumor types (Figure 2). Common upregulated genes were associated with cell death, necrosis, and homeostasis (Figure 3B). This included TNFSF11 (FC 3.00, FDR = 0.008) and LTB (FC 2.68, FDR < 0.001) which are important for tumor necrosis factor receptor binding. There were three common upregulated genes that had $\log_2$FC ≥2.00 in gene expression. These genes were CCL21 (FC 10.38, FDR <0.001), CD19 (FC 5.07, FDR <0.001), and FCER2 (FC 4.78, FDR <0.001; Table 3). Notably, there was a common upregulation of genes related to chemotaxis, which additionally included CCL19 (FC 3.19, FDR <0.001), TNFSF11 (FC 3.00, FDR = 0.008), and CXCL13 (FC 2.97, FDR <0.001).

3.5 | Common downregulated genes: ALN compared to PBC

When gene expression of the ALN for ER-positive PBC was compared to that of TNBC, 115 genes were downregulated in both tumor types (Figure 2). Common downregulated genes were those of migration, degradation of extracellular matrix, and invasion (Figure 3B), which include downregulated genes: MMP2 (fold change (FC) −2.1, FDR < 0.01), MMP3 (FC −2.1, $P < .01$), COL17A1 (FC −3.2, FDR < 0.01), COL6A6 (FC −2.5, FDR < 0.01), and COL11A1 (FC −2.0, FDR < 0.01). Other common downregulated genes included FGF5 (FC −5.4, FDR < 0.01) which is a fibroblast growth factor related to cell survival, SFRP2 (FC −2.7, FDR < 0.01) which is has a role in regulating cell growth as modulators of Wnt signaling,

and WNT2 (FC −2.3, FDR < 0.01) which is a signaling protein related to oncogenesis. There were 8 common downregulated genes that had log_2FC <−2.00 in gene expression which include FGF5 (FC 0.02, FDR < 0.001), COL17A1 (FC 0.10, FDR = 0.005), SFRP2 (FC 0.16, FDR < 0.001), COL6A6 (FC 0.17, FDR < 0.001), WNT2 (FC 0.20, FDR < 0.001), MMP2 (FC 0.23, FDR < 0.001), MMP3 (FC 0.24, FDR < 0.001), and COL11A1 (FC 0.24, FDR < 0.001). A notable feature of common downregulated genes was the regulation of cell microenvironment interaction, which included several additional Matrix Metallopeptidases (MMP 7, MMP 11, and MMP14) and Collagens (COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, and COL6A6). Reactome analysis showing gene coverage for significant common genes shows involvement of several pathways including the immune system and signal transduction (Figure 3A).

3.6 | Unique upregulated and downregulated genes: ALN compared to ER-positive PBC

Compared with the ER-positive PBC, ALN metastasis had 251 uniquely upregulated genes (Figure 2). There were 12 unique upregulated genes that had log_2FC ≥ 2.00 in gene expression which include CD5 (FC 2.75, FDR < 0.001), KLRB1 (FC 2.59, FDR < 0.001), CXCL9 (FC 2.59, FDR < 0.001), CD79A (FC 2.56, FDR < 0.001), and

TABLE 3 Five most common upregulated and downregulated genes with at least a two log_2 fold change in gene expression in lymph node metastasis compared with PBC

Estrogen receptor-positive				Triple-negative breast cancer			
Gene	Log_2 fold change	Fold change	FDR	Gene	Log_2 fold change	Fold change	FDR
CCL21	4.52	22.92	<0.001	CCL21	3.38	10.38	<0.001
CCL19	3.07	8.37	<0.001	CACNA2D3	3.03	8.16	0.003
CD19	3.02	8.08	<0.001	CD19	2.34	5.07	<0.001
LTB	2.78	6.85	<0.001	FCER2	2.26	4.78	<0.001
FCER2	2.77	6.82	<0.001	PGC	2.24	4.72	<0.001
KRT17	−3.22	0.11	<0.001	COL6A6	−2.60	0.17	<0.001
FGF5	−3.53	0.09	<0.001	SFRP2	−2.70	0.16	<0.001
SFRP2	−3.54	0.09	<0.001	COL17A1	−3.29	0.10	0.004
KRT14	−3.62	0.08	<0.001	CHRNA1	−3.94	0.07	0.023
COL17A1	−3.95	0.06	<0.001	FGF5	−5.36	0.02	<0.001

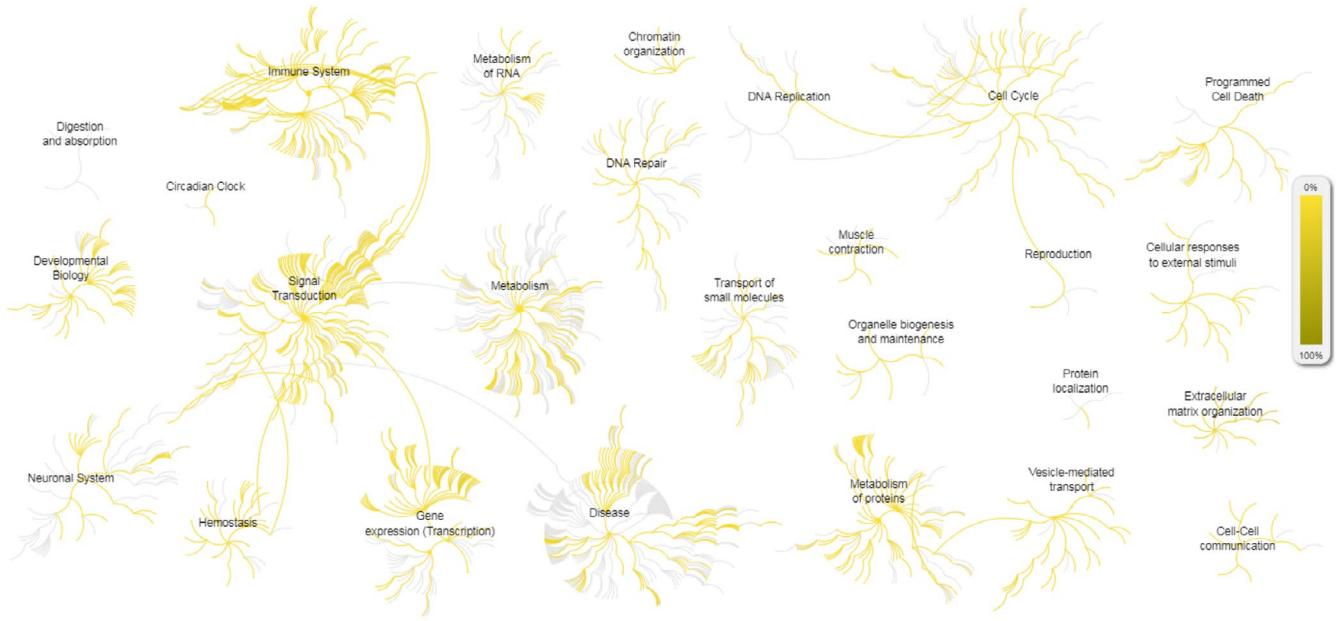

FIGURE 3 Reactome Analysis and Canonical Pathways of breast cancer cells in the axillary lymph node (ALN) compared to the primary breast cancer (PBC) for common genes in ER-positive compared with TNBC. A, Reactome analysis of common genes colored by gene coverage. B, Canonical pathways of common genes by biologic function. Activation *z*-scores represent the activation (positive values in orange) or inhibition (negative values in blue) of cellular functions

FIGURE 3 (Continued)

LAT (FC 2.44, FDR < 0.001). Compared with the ER-positive PBC, ALN metastasis had 210 uniquely downregulated genes. There were 6 unique downregulated genes that had $\log_2$FC <−2.00 in gene expression which include KRT14 (FC −3.6, FDR < 0.001), KRT5 (FC −3.06, FDR < 0.001), SERPINE1 (FC −2.31, FDR < 0.001), KLK4 (FC −2.07, FDR < 0.001), MIA (FC −1.07, FDR ,0.001, and EGR1 (FC −2.02, FDR < 0.001).

3.7 | Unique upregulated and downregulated genes: ALN compared to TNBC

Compared with TNBC, ALN metastasis had 26 uniquely upregulated genes (Figure 2). There were only 2 unique upregulated genes that had $\log_2$FC ≥ 2.00 in gene expression which include CACNA2D3 (FC 8.16, FDR = 0.003) and PGC (FC 4.72, FDR < 0.001). Compared with TNBC, ALN metastasis had 29 uniquely downregulated genes. CHRNA1 (FC 0.07, FDR = 0.023) was the only uniquely downregulated gene that had $\log_2$FC <−2.00 in gene expression.

3.8 | Changes in gene expression by tumor subtype

There were no genes that were upregulated in ER-positive PBC that were downregulated in TNBC. Similarly, there were no genes that were downregulated in ER-positive PBC that were upregulated in TNBC.

4 | DISCUSSION

Gene expression studies for breast cancer contribute to understanding breast cancer biology and the metastatic process. Additional contributions include drug development and the development of prognostic gene expression signatures.[14] Early studies on gene expression of primary breast cancer compared PBC to paired distant organ metastasis and often showed similar gene expression and identified potential markers for breast cancer metastasis.[15-17] More recently, several studies have analyzed gene expression of primary breast cancer compared with paired lymph node metastasis. While these studies had variable results on important genes for tissue modeling and cell cycle regulators, they did not evaluate the breast cancer subtype and receptor profiles used which may have meaningful differences in gene expression.[18-21]

Smeets et al analyzed 96 pairs of PBC and paired lymph node metastasis for patients with estrogen receptor-positive invasive ductal carcinoma. In the 241 genes that they evaluated, they found that genes related to apoptosis, protein phosphorylation, and zinc chelation had changes in gene expression compared to patients without lymph node metastasis.[22] They demonstrated an upregulation of genes related to apoptosis in the lymph node-positive group. Our study similarly showed an upregulation of common differentially expressed genes in both ER-positive and TNBC related to cell death and necrosis. Genes related to chemotaxis were commonly upregulated in both ER-positive and TNBC, similar to our previous findings.[8] When examining common downregulated genes in ER-positive and TNBC, genes related to migration, survival, and cell microenvironment interaction showed underexpression.

Most genomic expression analysis studies on TNBC to metastatic disease have been of distant metastases, and rarely lymph node metastases.[23-25] Our study not only compares common gene expression in ER-positive and triple-negative IDC that has metastasized to axillary lymph nodes, but also evaluates genes that are uniquely expressed in each PBC receptor subtype. Two genes, CACNA2D3 and PGC, are uniquely upregulated in TNBC, and a single gene CHRNA1 is uniquely downregulated. These genes that are not differentially expressed in ER-positive tumors may help to differentiate the tumor subtypes using gene expression and be important prognostic indicators unique to TNBC. This finding highlights the importance of gene expression analysis by receptor subtype.

Another important finding of our study is that no genes that were upregulated in ER-positive PBC were downregulated in TNBC, and the converse is similarly true. This finding emphasizes that although there are unique patterns in gene expression for ER-positive and TNBC, there are importantly a significant number of genes that are similarly up- or downregulated.

Our study has several limitations. The sample size was small because it is challenging to locate patients at with TNBC who were not treated with neoadjuvant therapy. A micro-dissection method for tissue isolation limits contamination of lymphoid cells and includes primarily tumor cells. A limitation of Next Generation Sequencing includes variable results due to differences in the number of genes evaluated and the heterogeneity of breast and metastatic tumor samples.

5 | CONCLUSION

Although ER-positive PBC and TNBC have a distinct gene expression profiles and distinct changes from PBC to ALN metastases, a significant number of genes are similarly up- or downregulated. Understanding the role of these common genomic changes may provide clues to understanding the metastatic process itself.

ACKNOWLEDGEMENTS

Armando E. Giuliano is supported by the Fashion Footwear Charitable Foundation of New York, Inc, the Margie and Robert E. Petersen Foundation, and Linda and Jim Lippman. Xiaojiang Cui is supported by National Institutes of Health (2R01CA151610) and Department of Defense (W81XWH-18-1-0067), Samuel Oschin Cancer Institute Discovery Fund Award, and Community Outreach and Engagement Developmental Fund Award. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

CONFLICT OF INTEREST

All authors have no conflicts of interest or other disclosures.

ORCID

Marissa K. Srour https://orcid.org/0000-0001-9206-2716

REFERENCES

1. Howlader N, Altekruse SF, Li CI, et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. *J Natl Cancer Inst*. 2014;106(5):1-8.
2. Dings PJ, Elferink MA, Stobbe LJ, de Wilt JH. The prognostic value of lymph node ratio in node-positive breast cancer: a Dutch nationwide population-based study. *Ann Surg Oncol*. 2013;20(8):2607-2614.
3. Liao GS, Chou YC, Golshan M, et al. Prognostic value of the lymph node ratio in breast cancer subtypes. *Am J Surg*. 2015;210(4):749-754.
4. O'Brien KM, Cole SR, Tse CK, et al. Intrinsic breast tumor subtypes, race, and long-term survival in the Carolina breast cancer study. *Clin Cancer Res*. 2010;16(24):6100-6110.
5. Jang N, Choi JE, Kang SH, Bae YK. Validation of the pathological prognostic staging sytem proposed in the revised eighth edition of the AJCC staging manual in different molecular subtypes of breast cancer. *Virchows Arch*. 2019;474(2):193-200.
6. Yates LR, Desmedt C. Translational genomics: practical applications of the genomic revolution in breast cancer. *Clin Cancer Res*. 2017;23(11):2630-2639.
7. Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol*. 2013;31(31):3997-4013.
8. Srour MK, Gao B, Dadmanesh F, et al. Gene expression comparison between primary triple-negative breast cancer and paired axillary and sentinel lymph node metastasis. *Breast J*. 2020;26(5):904-910.
9. Schwartz M, Kremer J.Verio Laboratory Services: HTG EdgeSeq oncology biomarker panel. 2018.
10. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 2010;26(1):139-140.
11. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Stat Soc: Ser B (Methodol)*. 1995;57:289-300.
12. "Reactome: Pathway Analysis Report". Version 68. https://reactome.org/PathwayBrowser/#/ANALYSIS=MjAxOTA0MzAxNTMwNDBfMTQz. Accessed April 30, 2019.
13. Fabregat A, Sidiropoulos K, Garapati P, et al. The reactome pathway knowledgebase. *Nucleic Acids Res*. 2016;44(D1):D481-D487.
14. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature*. 2000;406(6797):747-752.
15. Lee JY, Park K, Lee E, et al. Gene expression profiling of breast cancer brain metastasis. *Sci Rep*. 2016;6:28623.
16. Weigelt B, Glas AM, Wessels LFA, Witteveen AT, Peterse JL, van't Veer LJ. Gene expression profiles of primary breast tumors maintained in distant metastases. *Proceedings of the National Academy of Sciences of the United States of America*. 2003;100(26):15901-15905.
17. Bell R, Barraclough R, Vasieva O. Gene expression meta-analysis of potential metastatic breast cancer markers. *Curr Mol Med*. 2017;17:200-210.
18. Hao X, Sun B, Hu L, et al. Differential gene and protein expression in primary breast malignancies and their lymph node metastases as revealed by combined cDNA microarray and tissue microarray analysis. *Cancer*. 2004;100(6):1110-1122.
19. Weigelt B, Wessels LFA, Bosma AJ, et al. No common denominator for breast cancer lymph node metastasis. *Br J Cancer*. 2005;93(8):924-932.
20. Suzuki M, Tarin D. Gene expression profiling of human lymph node metastases and matched primary breast carcinomas: clinical implications. *Mol Oncol*. 2007;1(2):172-180.
21. Feng Y, Sun B, Li X, et al. Differentially expressed genes between primary cancer and paired lymph node metastases predict clinical outcome of node-positive breast cancer patients. *Breast Cancer Res Treat*. 2007;103(3):319-329.
22. Smeets A, Daemen A, Vanden Bempt I, et al. Prediction of lymph node involvement in breast cancer from primary tumor tissue using gene expression profiling and miRNAs. *Breast Cancer Res Treat*. 2011;129(3):767-776.
23. Ding L, Ellis M, Li S, et al. Genome remodeling in a basal-like breast cancer metastasis and xenograft. *Nature*. 2010;464(7291):999-1005.
24. Hoadley KA, Siegel MB, Kanchi KL, et al. Tumor evoluation in two patients with basal-like breast cancer: a retrospective genomics study of multiple metastases. *PLoS Med*. 2016;13(2):1-20.
25. Zhou L, Gao HF, Liu DS, Feng JY, Gao DD, Xia W. Gene expression profiling of brain metastatic cell from triple negative breast cancer: understanding the molecular events. *Gene*. 2018;640:21-27.

How to cite this article: Srour MK, Qu Y, Deng N, et al. Gene expression comparison between primary estrogen receptor-positive and triple-negative breast cancer with paired axillary lymph node metastasis. *Breast J*. 2021;00:1–9. https://doi.org/10.1111/tbj.14119