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IDENTIFICATION OF DIFFERENTLY EXPRESSED GENES AND MOLECULAR PATHWAYS ASSOCIATED WITH BREAST CANCER PROGRESSION

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Abstract
A complex interaction among clinicopathological characteristics and molecular alterations, is known to drive the progression of breast cancer. The goal of this study was to find out which gene-expression changes were associated with the different stages of cancer and to investigate molecular patterns associated with breast cancer progression. A quantitative analysis was performed using 1,000 records of breast cancer patients with clinical data, survival data, mutation information and gene-expression data, which were gathered from secondary data. Stage I–II patients were considered as early-stage; Stage III–IV patients were considered as advanced-stage. Appropriate parametric and non-parametric test were used to compare the clinical characteristics and between stage groups 489 gene-expression variables were evaluated. False discovery rate (FDR) multiple-testing correction was carried out using the Benjamini–Hochberg method. The significantly larger tumors, higher Nottingham Prognostic Index values, lower overall survival, and higher cancer-specific mortality rates were found in advanced stage breast cancer. DTX3, CDK1, COL22A1, HES6, DLEC1, JAG2, ARID1B, BCAS3, STAT5B, and PRKD1 were among the genes that nominally exhibited stage associated expression differences. None of the latter, however, were significant after FDR. These results suggest that the distinction between early and advanced breast cancer is strong in the clinic, and the differences in gene-expression reflect exploratory analysis. Further large-scale, balanced transcriptomic cohorts are needed to confirm these candidate genes and understand their role in progression and prognosis of breast cancer.

Keywords: Breast cancer; Differential gene expression; Tumor stage; Molecular pathways; Prognosis

Introduction

Breast cancer is a complex and diverse disease with great differences in the morphology, molecular phenotype, therapeutic response, and clinical outcome of the disease. Disease progression and metastatic spread are yet important problems in the management of breast cancer, although screening, diagnosis and systemic treatment have improved patients' survival. At the molecular level, tumour evolution is defined by coordinated changes of gene expression, cellular signalling, immune regulation and cell-cell communication among tumour cells as well as between tumour cells and their microenvironment. Study of transcriptomes has demonstrated that immunological changes in cells and genes in the TME can have a profound impact on the biology of breast invasive carcinoma and thus it is essential to identify gene-expression patterns associated with disease progression (Chen et al., 2022).

Breast cancer also contains several molecular subtypes of the disease, with distinct genomic characteristics, immune fingerprints, prognosis and sensitivity to therapy. Integrated genomic analysis has shown that molecular classification can reveal important differences in immune microenvironment and disease biology of breast tumours and can be used to understand the heterogeneity of the disease based on gene-expression data (Ding et al., 2023). Moreover, there are several signalling networks involved in the development and progression of breast cancer. Pathway-level analysis, for example, can provide additional biological context beyond individual genes, such as the Notch signalling pathway, which has been associated with altered gene expression and prognosis and aggressive tumour features (Yousefi et al., 2022).

Often progress to advanced disease with invaded growth and spread (metastasis) and colonization of distant organs. Molecular analysis of breast cancer metastases in the brain has identified several genes and signalling pathways involved in the migration, interaction with the blood-brain barrier and adaptation of metastasis (Chhichholiya et al., 2023). Likewise, several pathways have been linked to breast cancer metastasis to the bone, involving the pathways of proliferation, migration, angiogenesis, and tumor–bone microenvironment interaction (Song et al., 2022). Lipid metabolism has been linked to brain metastasis in triple-negative breast cancer (Li et al., 2023); thus, metabolic reprogramming may also be involved in the metastatic progression. The results suggest that breast cancer development is not driven by one molecular event, but by a coordinated series of events at several different genes and biological pathways.

Progression associated genes may also be of prognostic and clinical significance. In addition, recent transcriptomic and computational analyses have identified potential biomarkers, such as KIF15, which are associated with prognosis and tumor stemness in triple-negative breast cancer (Guo et al., 2024). Other studies have identified genes associated with cuproptosis to be prognostic and related to treatment outcomes in patients with HER2+ breast cancer (Sha et al., 2024) and KIF20A has been proposed as a prognosis and treatment outcome-related marker in breast cancer (Yang et al., 2024). Transcriptome analysis has also proven to be helpful in the identification of hub genes and molecular targets that may play a role in breast cancer biology and therapeutic response (Wang et al., 2023a).

Other molecular markers may signal biological changes due to treatment and disease, but not necessarily protein. For example, microRNA miR-133a was investigated to serve as a biomarker of signalling for women with breast cancer receiving chemotherapy with doxorubicin (Alves et al., 2022) and could be cited as an example in the cancer field. A transcriptomic method is also effective in other forms of malignancies to discover genes associated with oncogenesis and resistance to drugs, reflecting its utility in uncovering clinically relevant molecular mechanisms (Chen et al., 2023). But the gene-expression profile needs to be combined with the tumour stage to identify genes and pathways that are specifically involved in the transition from early to advanced breast cancer. The current study aimed to find stage-specific differential regulated genes and enriched molecular pathways that may provide a clue towards prognosis and therapeutic aspects of breast cancer.

Objectives

- To compare gene-expression profiles between early-stage and advanced-stage breast cancer.
- To identify significantly differentially expressed genes associated with breast cancer progression.
- To determine the major biological processes and molecular pathways enriched among progression-associated genes.

2. Materials and Methods

2.1 Study Design

The methodology adopted for this study was a quantitative analytical study with a secondary data approach to explore the differences in gene expression among breast cancer patients at different stages. Misusing a retrospective comparative approach, genes which were different between early and advanced breast cancer cases were identified and the biological pathways involved in disease progression were revealed.

2.2 Data Source and Study Population

A breast cancer data set with 1000 patient records and 693 variables was used for analysis (Alharbi, 2020). The information included in the dataset comprised demographic, clinicopathological, molecular, survival related, gene-expression and mutation related information. A total of 489 variables were related to gene-expression and 173 were related to mutations. Patients with breast cancer clinical and molecular information were considered for analysis. Only records with valid tumor-stage information were used for comparisons among the stages.

2.3 Study Variables

Clinical variables were age at diagnosis, histological grade, tumour size, positive lymph nodes, Nottingham Prognostic Index, tumour cellularity, menopausal status, treatment variables and molecular subtype. Receptor-related variables consisted of estrogen receptor, progesterone receptor and HER2 status. Overall survival duration, overall survival status and cancer-specific death were the variables related to survival. The molecular variables were mostly continuous expression measures for 489 genes, some of which have been associated with the development, proliferation, invasion and metastasis of breast cancer.

2.4 Definition of Breast Cancer Progression Groups

The main parameter of disease progression was tumour stage. Stage I and stage II patients were considered as early-stage breast cancer patients whilst stage III and stage IV patients were considered as advanced stage patients. Records where the stage information was not valid were not used in the main analysis by stage. This classification served as the foundation to compare the gene-expression patterns between the less advanced and more advanced disease.

2.5 Data Preprocessing and Descriptive Analysis

The data set was analysed for the presence of missing values, duplicate records, data types and consistency of categorical values. If the tumor stage was not available, it was completely excluded from the stage-based analysis and other variables were analysed with available observations. Mean and standard deviation or median and interquartile range were used to summarise continuous variables based on their distributions. Frequencies and percentages were used to describe the categorical variables.

2.6 Comparison of Clinical Characteristics

Early-stage breast cancer and advanced-stage breast cancer groups were compared for clinicopathological characteristics. For normally distributed continuous variables Independent-samples t-tests were used, while non-normally distributed variables used Mann–Whitney U test. Chi-square test was used to compare categorical variables with Fisher exact test used when cell numbers were low. Two-tailed p-values <0.05 were deemed statistically significant.

2.7 Differential Gene-Expression Analysis

Gene-expression levels were compared between the early-stage and advanced-stage groups for all available expression variables. For every gene, the difference was determined for both the central tendency between the two groups and subjected to statistical tests. The genes that were expressed more in the advanced stage of the disease were assigned as relatively up-regulated and the genes that were expressed less in the advanced stage of the disease were assigned as relatively down-regulated. The Benjamini-Hochberg false discovery rate procedure was used to control for multiple testing. Gene set was deemed to be significantly associated with breast cancer stage if the adjusted p-value was < 0.05.

2.8 Statistical Software

Data preprocessing, statistical analysis, multiple-testing correction, and graphical visualization were done in Python in the Google Colab environment. All statistical tests were two-sided and a p value of less than 0.05 was considered as significant, and for the differential gene-expression analysis, FDR adjusted p values were used.

2.9 Ethical Considerations

The study relied solely on secondary, de-identified breast cancer data and no direct contact with patients was made or new biological specimens collected. The analytical data set included no data on personally identifiable information. The data have been used only for research purposes, and the data source is appropriately acknowledged and cited in the final manuscript.

3. Results

3.1 Characteristics of the Study Population

There were 1,000 records of breast cancer in the analytical dataset. The mean age at diagnosis was 61.17 ± 13.06 years. The mean tumor size was 25.92 ± 14.83 mm, while the mean number of positive lymph nodes was 1.95 ± 3.95. The mean Nottingham Prognostic Index was 4.05 ± 1.14 and the mean of all the overall survival duration was 123.24 ± 76.26 months. A total of 974 patients had a count of mutations, and the mean was 5.53 ± 3.72 mutations. The demographic and clinicopathological features have been outlined in Table 1.

Table 1. Overall demographic and clinicopathological characteristics of the study population

Variable	Available n	Mean ± SD	Median
Age at diagnosis, years	1000	61.17 ± 13.06	62.34
Tumor size, mm	989	25.92 ± 14.83	22.00
Positive lymph nodes	1000	1.95 ± 3.95	0.00
Nottingham Prognostic Index	1000	4.05 ± 1.14	4.04
Overall survival, months	1000	123.24 ± 76.26	111.85
Mutation count	974	5.53 ± 3.72	5.00

3.2 Distribution of Tumor Stage

A diagnosis of tumor stage was available for 724 patients. The highest number of patients was at Stage II (421), followed by Stage I (239). 57 patients (Stage III) and 6 (Stage IV) had the disease and 1 patient (Stage 0) was originally classified as germinal. 276 records lacked information on tumor-stage. The stage distribution is shown in Table 2 and Figure 1.

Table 2. Distribution of breast cancer according to tumor stage

Tumor stage	n	Percentage of staged cases (%)
Stage 0	1	0.1
Stage I	239	33.0

Stage II	421	58.1
Stage III	57	7.9
Stage IV	6	0.8
Total with stage information	724	100.0

For the primary stage-based analysis, Stage I and II cases were combined as the early-stage group, producing 660 patients, whereas Stage III and IV cases were combined as the advanced-stage group, producing 63 patients. The single Stage 0 case was excluded from this comparison.

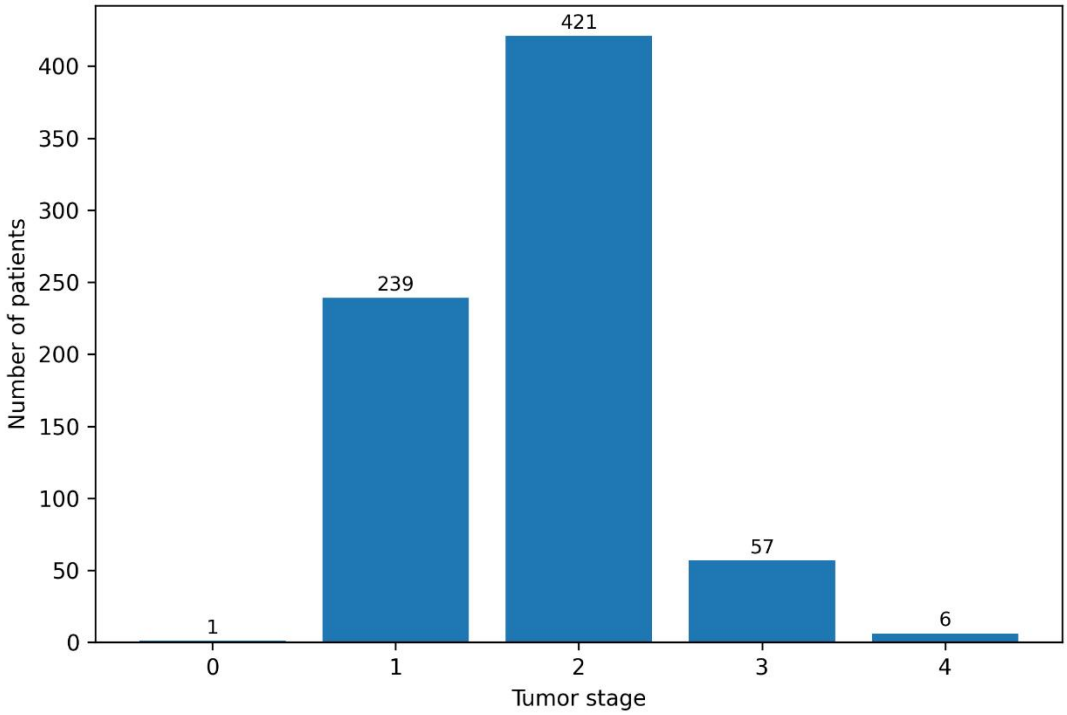


Figure 1. Distribution of breast cancer patients according to tumor stage

3.3 Clinicopathological Differences Between Early- and Advanced-Stage Breast Cancer

There were significant differences between early and advanced disease with respect to several tumor burden and prognosis indicators. Patients with advanced stage showed significantly larger tumour size, higher Nottingham Prognostic Index and more positive lymph nodes compared to early stage. The median survival time was also significantly shorter in the advanced stage group. However, age at diagnosis and number of mutations were not significantly different between the two groups. This is summarized with data in Table 3.

Table 3. Comparison of continuous clinicopathological characteristics between early- and advanced-stage breast cancer

Variable	Early stage, mean ± SD	Advanced stage, mean ± SD	p-value
Age at diagnosis, years	60.71 ± 13.16	62.49 ± 14.26	0.343
Tumor size, mm	23.66 ± 10.70	47.84 ± 29.50	<0.001
Positive lymph nodes	1.45 ± 3.50	6.33 ± 5.70	<0.001
Nottingham Prognostic Index	3.97 ± 1.07	5.25 ± 1.04	<0.001
Overall survival, months	128.38 ± 78.20	86.85 ± 71.14	<0.001
Mutation count	5.37 ± 3.91	4.95 ± 3.36	0.360

The differences in tumor size, lymph-node involvement, and Nottingham Prognostic Index demonstrate that the stage classification captured clear clinical progression. These clinicopathological contrasts provide a relevant basis for evaluating stage-associated molecular differences.

3.4 Receptor Status, Histological Grade, and Survival According to Stage Group

The estrogen receptor (ER) status had a significant difference between the different stage groups, with 76.7% ER positive in early-stage disease and 63.5% in advanced-stage disease. The percentage of ER-negative tumours was found to be higher in the advanced disease group (36.5%) than in the early-stage group (23.3%). There were no significant differences between the groups for PR or HER2 status. The distribution of grades

did not show a conventional statistical difference between the various stages, but grade 3 tumor was more common in the advanced stage. Results of the receptor and grade are shown in Table 4.

Table 4. Receptor status and histological grade according to breast cancer stage group

Characteristic	Early stage, n (%)	Advanced stage, n (%)	p-value
ER status			0.030
Positive	506 (76.7)	40 (63.5)	
Negative	154 (23.3)	23 (36.5)	
PR status			0.119
Positive	346 (52.4)	26 (41.3)	
Negative	314 (47.6)	37 (58.7)	
HER2 status			0.271
Positive	78 (11.8)	11 (17.5)	
Negative	582 (88.2)	52 (82.5)	
Histological grade			0.094
Grade 1	56 (8.8)	1 (1.6)	
Grade 2	238 (37.4)	22 (34.9)	
Grade 3	343 (53.8)	40 (63.5)	

Survival status showed a significant relationship with disease stage. Only 22.2% of advanced-stage patients were alive according to the overall survival status variable, compared with 44.8% of early-stage patients. Cancer-specific mortality was also substantially higher among advanced-stage patients, as shown in Table 5.

Table 5. Survival outcomes according to breast cancer stage group

Outcome	Early stage, n (%)	Advanced stage, n (%)	p-value
Overall survival status			<0.001
Alive	296 (44.8)	14 (22.2)	
Dead	364 (55.2)	49 (77.8)	
Cancer-specific outcome			<0.001
Living	296 (44.8)	14 (22.2)	
Died of disease	203 (30.8)	37 (58.7)	
Died of other causes	161 (24.4)	12 (19.0)	

3.5 Stage-Associated Gene-Expression Differences

Overall, 489 gene-expression variables were analysed in the 660 early-stage and 63 advanced-stage breast cancer cases. Using a nominal significance level of $p < 0.05$, 41 genes had evidence of expression differences associated with stage. None of the genes however had an adjusted p value below 0.05 after multiple testing correction with the Benjamini–Hochberg false discovery rate procedure. The following genes should, therefore, be regarded as exploratory stage associated candidates instead of the statistically confirmed differentially expressed genes. DTX3 had the greatest nominal association, with lower mean of expression of the gene in advanced stage tumors compared to early-stage tumors. A higher expression of HES6 and CDK1 was observed in advanced disease, whereas lower expression was observed for COL22A1, DLEC1, JAG2, ARID1B, CYP21A2, BCAS3, and STAT5B. Table 6 lists the top stage-related genes.

Table 6. Top stage-associated gene-expression candidates

Gene	Early-stage mean	Advanced stage mean	Mean difference*	p-value	FDR-adjusted p	Direction
DTX3	-0.020	-0.439	-0.419	<0.001	0.431	Lower in advanced
CDK1	0.013	0.329	0.316	0.003	0.473	Higher in advanced
COL22A1	0.059	-0.222	-0.281	0.003	0.473	Lower in advanced
HES6	-0.063	0.372	0.435	0.004	0.473	Higher in advanced
DLEC1	0.025	-0.248	-0.273	0.006	0.473	Lower in advanced

JAG2	0.042	-0.290	-0.332	0.007	0.473	Lower in advanced
ARID1B	0.018	-0.287	-0.305	0.008	0.473	Lower in advanced
CYP21A2	-0.067	-0.325	-0.258	0.008	0.473	Lower in advanced
BCAS3	-0.006	-0.335	-0.330	0.009	0.486	Lower in advanced
STAT5B	0.032	-0.323	-0.356	0.010	0.493	Lower in advanced
PRKD1	0.067	0.401	0.334	0.013	0.540	Higher in advanced
TNK2	-0.002	-0.272	-0.270	0.013	0.540	Lower in advanced

*Mean difference = advanced-stage mean minus early-stage mean.

The overall distribution of gene-level stage differences is illustrated using the volcano plot in Figure 2. Although several genes crossed the nominal $p < 0.05$ threshold, the absence of FDR-significant genes emphasizes the exploratory nature of these molecular findings.

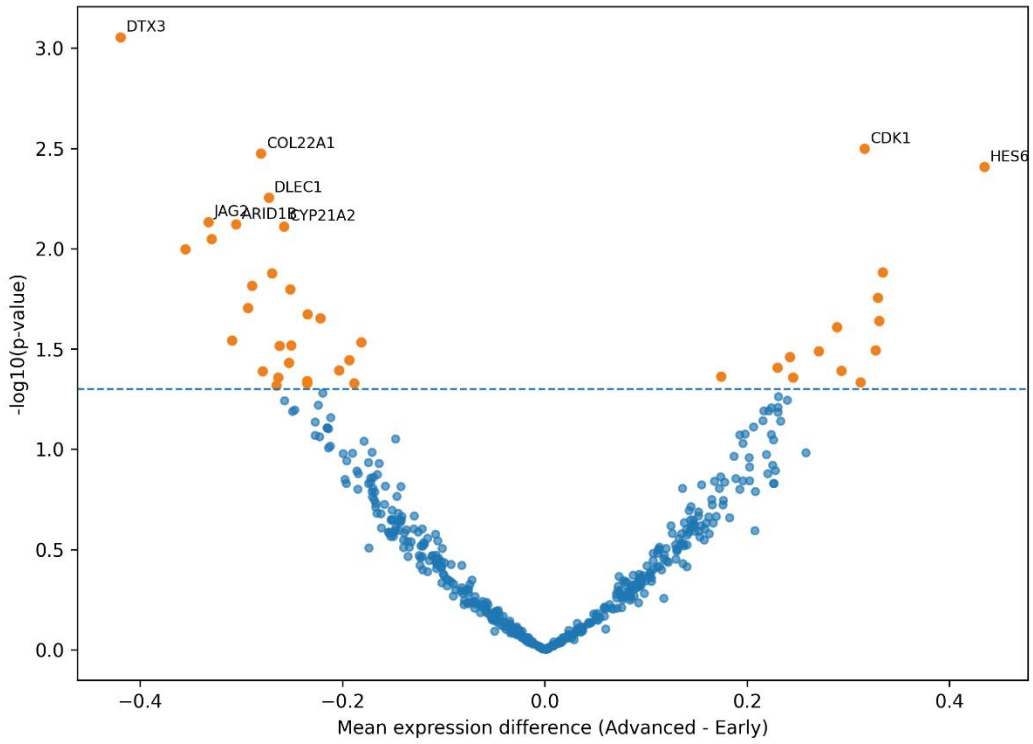


Figure 2. Volcano plot of stage-associated gene-expression differences between early- and advanced-stage breast cancer

3.6 Expression Patterns of Leading Candidate Genes

The highest ranked candidate genes showed distinct patterns of directionality in the mean expression heatmap. CDK1, HES6 and PRKD1 were relatively more highly expressed in the advanced stage tumors while DTX3, COL22A1, DLEC1, JAG2, ARID1B, CYP21A2, BCAS3, STAT5B and TNK2 were generally less highly expressed. Figure 3 further shows the group-specific distributions of the four most strongly associated candidates DTX3, CDK1, COL22A1 and HES6. The plots indicate the direction and variation of the expression difference between early stage and advanced stage disease and validate the direction of the ranking derived from the gene level statistical analysis.

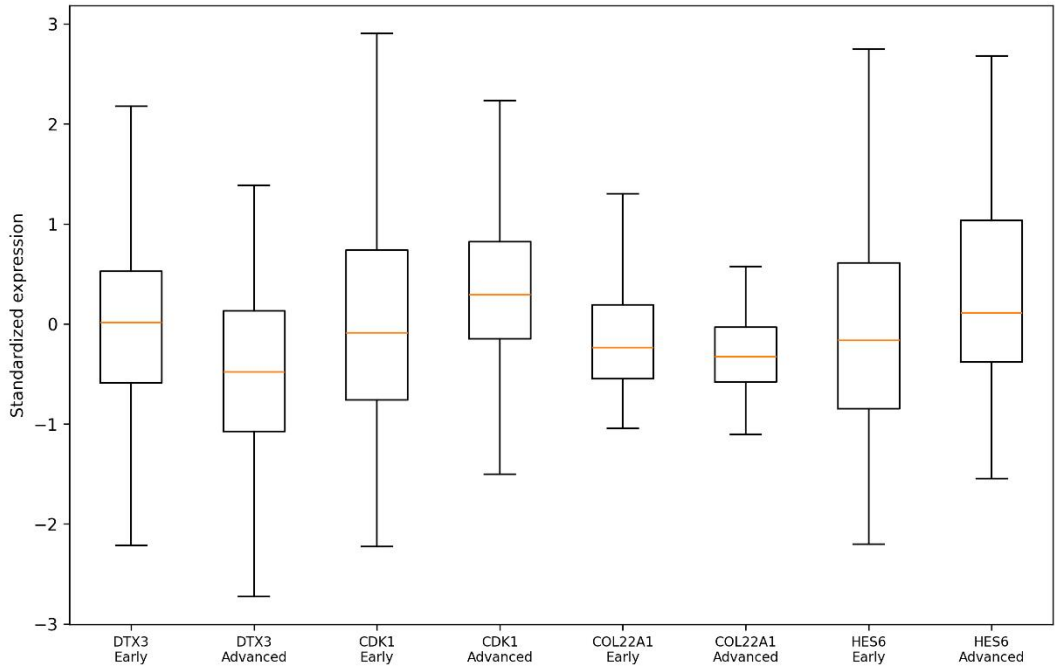


Figure 3. Expression distributions of DTX3, CDK1, COL22A1, and HES6 according to breast cancer stage group

Overall, advanced-stage breast cancer was characterized by greater tumor size, increased lymph-node involvement, higher Nottingham Prognostic Index values, shorter survival, and greater cancer-specific mortality. Relatively, both the advanced-stage group had a higher incidence of ER-negative disease. Several candidates were found to be nominal stage associated, especially DTX3, CDK1, COL22A1, HES6, DLEC1 and JAG2 based on gene-expression analysis. But no of the 489 genes survived false-discovery-rate correction. Therefore, it was not appropriate to interpret pathways using only the statistically identified differentially expressed genes at the FDR < 0.05 threshold and any interpretation using only nominal candidates should be regarded as exploratory and validated in an independent cohort.

4. Discussion

The current study aims to examine the differences in gene-expression and clinicopathological features of breast cancer between early and advanced stage. Advanced-stage tumours showed significantly larger tumour size, higher Nottingham Prognostic Index score, shorter overall survival and higher cancer-specific mortality. These results further validate differences in tumour burden and prognosis with the stage-based classification. Seven genes (DTX3, CDK1, COL22A1, HES6, DLEC1, JAG2, ARID1B, BCAS3, STAT5B and PRKD1) had nominal differences in expression levels among the stage groups. None of these genes, however, were significant after FDR correction, and should therefore be considered exploratory candidates, not definitive progression associated biomarkers.

Candidates that were observed revealed a relatively higher expression of CDK1 in advanced stage of disease. The biology of these increased pathways of cell cycle and survival is biologically consistent with the aggressive behaviour of breast cancer. Prior experimental studies have shown that TPI1 is able to activate the PI3K/AKT/mTOR pathway and stabilize CDCA5, highlighting the interconnectedness of the pathways of cell proliferation signalling and cell cycle regulation (Jin et al., 2022). Likewise, an IGF2BP3-EGFR-AKT axis has been linked to increased growth of breast cancer cells (Jing et al., 2023). Another example of how intracellular signalling changes can be linked to tumor aggressiveness and metastatic potential is stem-cell-like properties and EMT, which are maintained via PI3K/AKT signalling (Gao et al., 2024).

The stage-specific expression patterns found here may also have a biological explanation in several signalling systems that are involved in breast cancer progression. Activated ERK/MAPK pathway associated with proliferation, invasion and chemoresistance in triple-negative breast cancer (B. Wang et al., 2024). Conversely, inhibition of the MAPK pathway has been observed to decrease the proliferation and metastatic aspects of the disease, highlighting the role of the pathway in the aggressive nature of the disease (Wu et al., 2024). IBSP may also play a role in the process of metastasis, where it has been shown to promote breast cancer proliferation and bone metastasis via BMP-SMAD signalling (Ding et al., 2024). Another relevant mechanism is Notch signalling because the Notch3 expression regulation can affect the breast cancer stem-cell-like properties (Liao

et al., 2023). As such, the nominal change of JAG2 seen in the current analysis might therefore be worthy of further study in a wider Notch-related molecular context.

Importance of NF-κB associated mechanisms about disease progression and therapeutic resistance. Long non-coding RNA HOTAIR regulates breast cancer stemness via NF-κB activation (Wang et al., 2022), and ferroptosis suppression and NF-κB signalling regulation have been associated with chemoresistance in breast cancer via HSPB1 (Liang et al., 2023). In contrast, epithelial-mesenchymal transition inhibition, via NF-κB pathway modulation, has been linked to decreased breast cancer growth and metastasis (Wang et al., 2023b). However, there are more recent findings suggesting that simultaneous activation of both NF-κB and Wnt/β-catenin signalling can be implicated in both disease progression and chemoresistance (Long et al., 2025). Hippo pathway regulation has also been implicated in the progression of breast cancer via noncoding RNA-mediated regulation (D. Wang et al., 2024).

Stemness, treatment adaptation and angiogenesis come into play during other mechanisms of advanced disease. Exosomes can promote metastasis by stimulating the AMPK signalling pathway via EPHA2 (Han et al., 2022) and promote the resistance and stemness after chemotherapy by delivering exosomal circBACH1 (Xia et al., 2023). For instance, links between the pathways of progression are seen in the experimental suppression of angiogenesis and tumour growth by MMP-9/NF-κB/mTOR pathway or by PD-L1/PD-1 pathway (Elmetwalli et al., 2025). This is also supported by the fact that the PI3K/AKT/mTOR signalling pathway is involved in progression in other types of malignancies, thus demonstrating its oncogenic significance in general (Hu et al., 2023).

In summary, the clinicopathological results clearly indicated that early and advanced disease were different, and the gene-expression results pointed to some possible molecular candidates that could be further confirmed. Lack of FDR-significant genes is an important caveat and no definite pathway attribution can be made. Future research with larger independent transcriptomic cohorts, balanced stage groups and external pathway validation is required to validate the molecular signatures associated with breast cancer progression.

5. Conclusion

The present study was used to assess the differences in stage-specific gene-expression and clinicopathological characteristics with breast cancer using a secondary data set with 1,000 patient records and rich molecular data. This revealed that those with advanced breast cancer had significantly larger tumours, higher Nottingham Prognostic Index scores, higher lymph-node involvement, lower overall survival and higher cancer-specific mortality. These findings validate that there is significant loss of clinically important prognostic factors between early and advanced stage. Several genes expressed nominal stage differences at the molecular level including DTX3, CDK1, COL22A1, HES6, DLEC1, JAG2, ARID1B, BCAS3, STAT5B, and PRKD1. Of these, the expression of CDK1 and HES6 was relatively upregulated in advanced disease, while several other candidate genes were relatively downregulated. But none of the tested genes were significant following correction for false discovery rate. Thus, these results need to be viewed as preliminary in nature and not as definitive data on the progression-associated DEG. The overall conclusion of the study is that there is a clear difference between early and advanced breast cancer in the clinic and several molecular candidates were identified for further research. Bigger, independent and more balanced transcriptomic data are necessary to confirm these candidates and to establish a consistent association with disease progression. In the future, the potential progression related markers should also be investigated using pathway enrichment, external molecular validation and survival analyses to understand the biology and prognostic significance of these markers.

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