Eurasian Journal of Medicine and Oncology

ORIGINAL RESEARCH ARTICLE

Differential gene expression and gene ontology associated with breast cancer development and progression: A meta-analysis study

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Citation: El Aboudi N, Ouardi F, Ababou M, Abdelilah L, Elbiad O, Badaoui B. Differential gene expression and gene ontology associated with breast cancer development and progression: A meta-analysis study. *Eurasian J Med Oncol*. 2025;9(4):296-310. doi: 10.36922/EJMO025060025

Received: February 7, 2025

Revised: April 27, 2025

Accepted: May 6, 2025

Published online: November 10, 2025

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Abstract

Introduction: Breast cancer (BC) is heterogeneous and remains a major health priority. Robust biomarkers are needed to improve early detection and guide therapy.
Objective: This study investigates the molecular mechanisms underlying BC development (normal versus cancer) and progression (early versus late).
Methods: A meta-analysis of 51 microarray studies comparing BC and normal cells, as well as early- and late-stage BC cells, was conducted using microarray data obtained from the Gene Expression Omnibus and ArrayExpress databases. Five meta-analysis methods, each based on different statistical approaches, were applied, and the overlapping results were identified.
Results: A total of 3,362 and 95 differentially expressed genes (DEGs) associated with BC development and progression were identified. Among these DEGs, the upregulation of *COL10A1*, *COL11A1*, *TOP2A*, *CDK1*, *MMP11*, and *S100P* and the downregulation of *ADH1B*, *SFRP1*, *DST*, *LEP*, *ADIPOQ*, and *CHRDL1* were the most significantly associated with BC development. DEGs such as *DHTKD1* and *CBX3* (upregulated) and *MAP3K20* (downregulated) were found to be among the most significantly differentially expressed in BC progression. The top gene ontology terms enriched in BC development included regulation of signaling receptor activity and cytokine-mediated signaling pathway. In addition, the cellular macromolecule biosynthetic process and response to organic substances were significantly enriched in BC progression.
Conclusion: Many of the DEGs may serve as potential therapeutic targets for BC.

Keywords: Breast cancer; Meta-analysis; Microarray data; Differentially expressed genes; Early-stage cancer; Late-stage cancer; Gene ontology

1. Introduction

Breast cancer (BC) is the most frequently diagnosed cancer among women worldwide, corresponding to 23.8% of all cancers, affecting 2.3 million women each year, among

whom 665,684 succumb to death.[1] BC remains a major health problem and currently represents a top biomedical research priority. New diagnostic and therapeutic tools are required to improve the personalized management of each patient.

BC is a complex and heterogeneous disease[2] with varied molecular features, tumor characteristics, expression patterns, and responses to therapy. Perou *et al.*[3] established the mammary tumor classification system based on the analysis of gene expression. They classified BC into four molecular classes: luminal-like, basal-like, normal-like, and human epidermal growth factor receptor 2-positive BC.[3]

Early detection of BC is of considerable clinical importance, and it can be used to make treatment decisions while the tumor burden is still low and when patients are most likely to respond to adjuvant therapy.[4] BC markers that showed evidence of clinical utility and were recommended for use in practice include carcinoembryonic antigen, estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, urokinase plasminogen activator, plasminogen activator inhibitor 1, the tumor suppressor *p53*, cathepsin D, and multiparameter assays for gene expression.[5]

Although some genes were found to be responsible for the development of BC, none of the mentioned markers can diagnose early- or late-stage BC. Thus, there is a crucial need to identify new biomarkers that enable the detection of early molecular phases in BC carcinogenesis and facilitate targeted treatments to prevent relapses. This necessity has recently driven intense research efforts to discover robust clinical and pathological prognostic and predictive factors.

Recent microarray-based expression profiling studies have provided evidence that breast tumors of different grades have distinct genomic, transcriptomic, and immunohistochemical profiles.[6] DNA microarrays can assess the expression of thousands of genes simultaneously.[7] Specifically, gene-expression profiling can potentially aid in the identification of prognostic markers for different types of cancers and new therapeutic targets. It has also been established that tumors that remain localized and primary tumors that metastasize have different gene expression profiles.[8]

Many microarray studies have resulted in a significant increase in publicly available databases, such as the Gene Expression Omnibus (GEO) from the National Center of Biotechnology Information (NCBI) and the ArrayExpress database from the European Bioinformatics Institute.[9,10] From these databases, numerous studies related to relevant diseases or biological processes (BP) can be accessed, and the number of available studies continues to grow rapidly. The most widely known and frequently used platforms include the Affymetrix GeneChips (*in situ* synthesized oligonucleotide microarray) and the Illumina high-density bead arrays.[11]

Combining transcriptomic studies, through meta-analysis, for a disease or condition could increase sensitivity and validate conclusions. Meta-analysis is most commonly applied to identify differentially expressed genes (DEGs),[12] which may serve as biomarkers or be used as features in classification models or classifiers to further refine a clinically useful gene signature.[13]

Several meta-analysis studies have been conducted to identify DEGs involved in BC and other subtypes of BC. Karn *et al.*[14] combined 3,030 Affymetrix HG-U133A microarrays from primary BC samples, including 238 of their own samples and 2,792 samples from 22 different publicly available datasets.[14] Sotiriou *et al.*[15] analyzed microarray data from 189 invasive BC samples (GSE2990) and three published gene expression datasets of breast carcinomas.[15] In addition, a meta-analysis was conducted on six triple-negative BC (TNBC) microarray expression data sets collected from the NCBI GEO database to identify robust DEGs of TNBC.[16]

Other studies using a few microarrays have also highlighted DEGs in BC.[17,18] R programming (http://www.r-project.org) and the associated Bioconductor project have become one of the most widespread tools for bioinformatics and biostatistics in recent years. Many newly developed meta-analysis methods have been published, including the Bayesian approach,[19] Fisher inverse chi-square,[20] probability of expression,[21] meta differential expression through distance synthesis,[22] and RankProd.[23] Furthermore, gene ontology (GO) analysis has become a commonly used approach for functional studies of large-scale genomic or transcriptomic data.

To date, published meta-analyses have employed different statistical approaches, often leading to differing conclusions. In addition, many of these studies relied on preprocessed data, which may hinder the accuracy of the analysis. Hence, in the present study, we performed a large-scale meta-analysis combining different statistical approaches to compare the expression profiles in BC and normal breast tissues, and in early- and late-stage BC tissues using microarray data accessible through GEO and ArrayExpress databases.

2. Methods

2.1. Data collection

We used "breast cancer" as the keyword to search for genome-wide expression studies in the NCBI-GEO

(http://www.ncbi.nlm.nih.gov/geo/) and European Molecular Biology Laboratory-European Bioinformatics Institute ArrayExpress databases (https://www.ebi.ac.uk/arrayexpress/). We used the GEOquery and ArrayExpress R packages in the R software (version R 4.1.3) to download cell intensity files. Only studies utilizing the Affymetrix platform were selected to analyze DEGs in BC, because various gene-expression profiling platforms measure the same gene with differing precision, relative scales, and dynamic ranges.[24] Only datasets that met the inclusion criteria were included in the analysis. The inclusion criteria were studies employing expression profiling by array and studies with cell intensity files. Studies involving cell lines were excluded from the analysis. The following information was also extracted from each identified study: accession number, country, year, status (cancer or normal), stage, platform, number of probes, ethnic group, and author. All publicly available microarray datasets that corresponded to BC studies using the Affymetrix platform published before March 4, 2019, were included. A total of 51 microarray datasets that corresponded to 10,959 chips, including 5,497 cases and 5,462 controls, were considered in this study (Table S1).

2.2. Preliminary preprocessing of datasets

We used the arrayQualityMetrics R package to reanalyze the quality of each dataset, which was scored on the basis of spatial, boxplot, heatmap, and run-length encoding metrics. We removed any array that failed in at least two metrics, as performed in a previous study.[25] Normalization for each dataset was performed independently using the robust multi-array average (RMA) expression measure[26] given in the affy package. RMA uses a model-based background correction, quantile normalization, and probe-level summarization to standardize signal intensities and reduce technical noise.

Subsequently, we extracted the common genes across all datasets using the R merge function; the data where genes do not overlap were excluded. To perform the meta-analysis, each dataset must consist of a control group; hence, for datasets lacking a control group, we considered a common reference control group from the other datasets included in this study. Although standardized preprocessing and normalization procedures were applied, the integration of data from multiple sources may introduce batch effects and other forms of variability.

2.3. Meta-analysis of the microarray data

The aim of this study was twofold: (i) to identify the set of DEGs between BC and control tissues using the complete dataset and (ii) to identify the DEGs associated with early- versus late-stage BC. Early-stage BC was defined as arrays corresponding to stage T1, node-negative (N0), grade 1, and early or primary BC. Late stage included all arrays in stage T4, node-positive (N1), metastatic (M1), and grade 3 BC cases. Accordingly, we highlighted 21 datasets harboring 1,438 chips, of which 1,153 were early-stage BC and 127 were late-stage BC.

We used the R Meta-Analysis of MicroArray package (R-Bioconductor 3.7 in R version 3.5.2) to perform meta-analysis of microarray, focusing on the overlapping outputs among five different methods: (i) *p*-value combination method (*p*val), (ii) effect size combination method (Es), (iii) meta-analysis pattern matches (MAP-Matches), (iv) meta-analysis of ranked discovery datasets (METRADISC), and (v) *z*-statistic method.

The p-value combination methods applied in this study included Fisher's method,[27] Stouffer's method,[28] the minimum p-value approach,[29] and the maximum p-value approach.[30] These methods aggregate significance levels obtained from individual studies. Other methods combine the effect size from each dataset. Depending on the assumption of homogeneity, either a fixed-effect model (FEM) or a random-effect model (REM) was adopted. FEM model combines the effect size across all studies by assuming a simple linear model with an underlying true effect size plus a random error in each study, while REM[31] allows random effects for the inter-study heterogeneity in the model. MAP-Matches involves computing *t*-statistics for each pairwise class comparison in individual datasets, followed by the construction of a *t*-statistics matrix for pattern matching. METRADISC[32] is a rank-based method that estimates heterogeneity across datasets. The *z*-statistic method utilizes the posterior mean differential expression, weighted by variance. The method assumes a standard normal distribution due to the classic Bayesian probability calculation and derives a prior distribution of differential expression to assess significance.

The datasets were transformed to a special object class from the MetaArray package, where separate slots were designated for gene expression data matrices (expression profiles), clinical sample characteristics (BC [1] and normal [0]), and dataset identifiers.

Five meta-analysis approaches were employed to identify the DEGs, after which the overlapping genes were considered as the final output of the meta-analysis. The top 100 DEGs genes in BC development and progression were considered for further analysis.

2.4. Gene ontology analysis

Gene ontology analysis was performed for the two meta-analyses using the topGO R package to identify the most represented biological functions in the two datasets. The

topGO package was designed to work with various test statistics and algorithms, taking GO dependencies into account.[33] The DEGs were analyzed using the topGO package, which uses Fisher's test to identify significantly enriched GO terms.[34,35]

3. Results

3.1. DEGs in BC development and progression

A total of 22,268 probes present across all datasets were considered for the identification of DEGs. Five different meta-analysis methods (*p*val, Es, MAP-Matches, METRADISC, and *z*-statistic) were performed, resulting in the identification of 8,031, 11,765, 21,205, 20,304, and 9,102 DEGs, respectively ($p<0.05$). The obtained DEGs from each method were plotted into a Venn diagram (Figure 1A), which highlighted 3,362 common DEGs in BC across all meta-analyses.

From a total of 10,959 microarray datasets used in BC, a subset of 12,80 was associated with early- (1,153 arrays) and late-stage (127 arrays) BC and was used for the meta-analysis of BC progression. The DEGs identified through the meta-analysis were plotted into a Venn diagram (Figure 1B), resulting in 95 common DEGs among all the meta-analysis methods. The top DEGs in BC development (100 DEGs) and progression (95 DEGs) are presented in Tables S2 and S3, respectively.

Among these DEGs, Table 1 shows the highly differentially expressed Oncogenes and Tumor suppressor genes in BC development and progression.

3.2. Gene ontology functional enrichment analysis

DEGs from BC development were subjected to enrichment analysis using the topGO R package. The most enriched BP were regulation of signaling receptor activity, cytokine-mediated signaling pathway, response to drug, negative regulation of cell proliferation, leukocyte migration, organic hydroxy compound transport, positive regulation of cell proliferation, G-protein coupled receptor signaling pathway, receptor-mediated endocytosis, and response to glucocorticoid (Table 2).

The most enriched GO terms in BC progression included cellular macromolecule biosynthetic process, response to organic substance, response to endogenous stimulus, cell development, cellular protein localization, cellular macromolecule localization, nucleic acid metabolic process, cellular response to organic substance, transcription by RNA polymerase II, and organonitrogen compound biosynthetic process (Table 3).

We found 17 and eight DEGs highly implicated in GO terms corresponding to BC development and progression, respectively. Among those genes, some were involved in more than one GO term (Table 4). These genes are more likely to play a fundamental role in cancer development and progression, and particular attention should be given to them.

4. Discussion

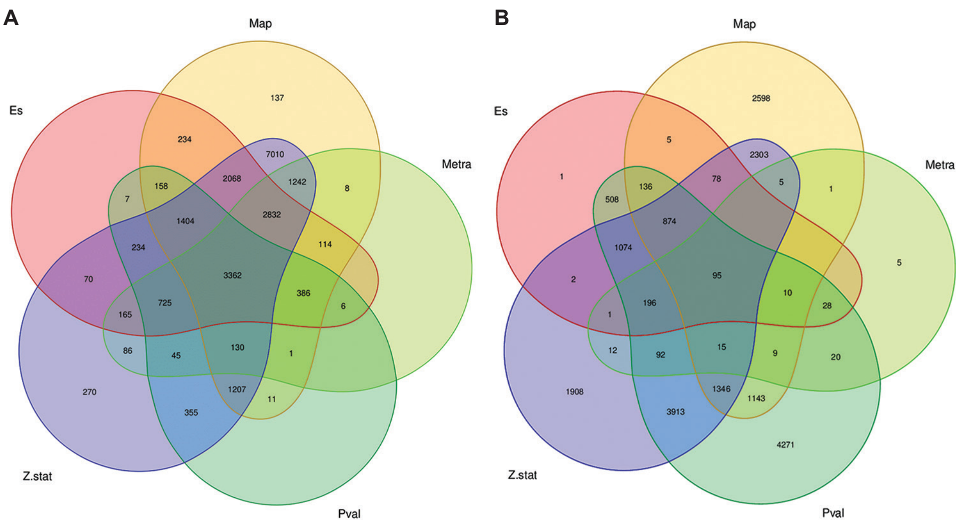


Figure 1. Venn diagrams of differentially expressed genes. (A) Venn diagram illustrating the differentially expressed genes in BC development using five statistical approaches. A total of 3,362 genes were common between the five meta-analysis methods. (B) Venn diagram illustrating the differentially expressed genes in BC progression using five statistical approaches. A total of 95 genes were common between the five meta-analysis methods.
Abbreviations: Es: Effect size combination method; Map: Meta-analysis pattern matches; Metra: Meta-analysis of ranked discovery datasets; Pval: *p*-value combination method; Z.stat: *z*-statistic method.

Table 1. Top differentially expressed oncogenes and tumor suppressor genes associated with breast cancer development and progression

Cases	Tumor suppressor genes	Oncogenes
Normal versus breast cancer	*CHL1*; *ABCA8*; *SFRP1*; *MME*	*COL10A1*; *COL11A1*; *ID4*; *CDK1*; *MELK*; *ATAD2*; *CEP55*; *HMMR*; *OXTR*
Early- versus late-stage breast cancer		*EVI5*; *SLC35A2*

Table 2. Gene ontology terms for breast cancer development

Gene ontology ID	Term	Annotated	Significant	Expected	Rank in classic Fisher	Classic Fisher	Classic KS	Elim KS
GO: 0010469	Regulation of signaling receptor activity	98	98	98	1	1	2.9e-10	2.9e-10
GO: 0019221	Cytokine-mediated signaling pathway	227	227	227	2	1	1.7e-10	7.7e-09
GO: 0042493	Response to drug	234	234	234	3	1	4.1e-09	9.6e-07
GO: 0008285	Negative regulation of cell proliferation	147	147	147	4	1	2.1e-08	1.7e-06
GO: 0050900	Leukocyte migration	130	130	130	5	1	1.7e-07	2.5e-06
GO: 0015850	Organic hydroxy compound transport	60	60	60	6	1	3.2e-06	3.2e-06
GO: 0008284	Positive regulation of cell proliferation	217	217	217	7	1	8.2e-14	4.9e-06
GO: 0007186	G protein-coupled receptor signaling pathway	179	179	179	8	1	2.7e-09	5.5e-06
GO: 0006898	Receptor-mediated endocytosis	97	97	97	9	1	5.9e-06	5.9e-06
GO: 0051384	Response to glucocorticoid	37	37	37	10	1	6.2e-06	6.2e-06

Note: Classic indicates that each gene ontology (GO) term was tested independently, not taking the GO hierarchy into account. Elim processes the GO terms by traversing the GO hierarchy from bottom to top.
Abbreviation: KS: Kolmogorov–Smirnov.

Table 3. Gene ontology terms for breast cancer progression

Gene ontology ID	Term	Annotated	Significant	Expected	Rank in classic Fisher	Classic Fisher	Classic KS	Elim KS
GO: 0034645	Cellular macromolecule biosynthetic process	37	37	37	1	1	0.0036	0.0036
GO: 0010033	Response to organic substance	21	21	21	2	1	0.0049	0.0049
GO: 0009719	Response to endogenous stimulus	12	12	12	3	1	0.0084	0.0084
GO: 0048468	Cell development	11	11	11	4	1	0.0155	0.0155
GO: 0034613	Cellular protein localization	21	21	21	5	1	0.0158	0.0158
GO: 0070727	Cellular macromolecule localization	21	21	21	6	1	0.0158	0.0158
GO: 0090304	Nucleic acid metabolic process	39	39	39	7	1	0.0174	0.0174
GO: 0071310	Cellular response to organic substance	18	18	18	8	1	0.0192	0.0192
GO: 0006366	Transcription by RNA polymerase II	18	18	18	9	1	0.0212	0.0212
GO: 1901566	Organonitrogen compound biosynthetic process	16	16	16	10	1	0.0306	0.0306

Note: Classic indicates that each gene ontology (GO) term was tested independently, not taking the GO hierarchy into account. Elim processes the GO terms by traversing the GO hierarchy from bottom to top.
Abbreviation: KS: Kolmogorov–Smirnov.

Table 4. Highly involved genes in the enriched gene ontology terms in breast cancer development and progression

Cases	Upregulated genes	Downregulated genes
Normal versus breast cancer	*INHBA*[a]; *CXCL10*[a]; *SPP1*[a]; *FN1*[a]; *CDK1*[a]; *ECT2*; *ARF1*; *RAB31*; *SDC1*	*LEP*[a]; *ADIPOQ*[a]; *SFRP1*[a]; *VEGFD*; *CXCL2*; *CD36*; *ANGPT1*; *TF*
Early- versus late-stage breast cancer	*CBX3*[a]; *DPM1*; *SHARPIN*; *RAC1*; *RNF10*; *YWHAZ*[a]; *XPO1*[a]; *DHTKD1*	-

Note: [a]genes implicated in more than one gene ontology term.

BC remains the most aggressive cancer, resulting in substantial mortality. The fight against BC represents a major challenge as its heterogeneity renders it difficult to understand. Failure to account for this variability can limit the generalizability and specificity of the findings. Gene expression studies combined with bioinformatics would permit the identification of biological features and target genes associated with BC, which could improve the understanding of molecular mechanisms shaping cancer development and progression.[36,37]

This study is a meta-analysis of publicly available transcriptomic datasets rather than an original clinical investigation. While this design enhances statistical power and generalizability by integrating data across diverse populations and platforms, it also limits the depth of clinical interpretation. The lack of patient-level clinical metadata restricts our ability to establish causal or prognostic relationships. As a result, further validation in prospective clinical studies is warranted.

Control samples were obtained from datasets different from the case samples. Integrating data from multiple sources introduces the potential for batch effects, despite normalization efforts. To our knowledge, we report herein the largest combined clinical and gene expression meta-analysis of BC using raw data and different statistical approaches to obtain the most pertinent results. Our meta-analysis integrated 51 BC microarray datasets and led to the identification of 3,362 and 95 DEGs for BC development and progression, respectively. These findings are discussed in the context of BP represented by the DEGs.

This study did not include external validation of the identified gene expression changes. The findings were derived from a single analytical pipeline without confirmation using experimental techniques. As a result, the reliability and reproducibility of the results are limited. Future studies should prioritize external validation to strengthen the biological and clinical relevance of the identified genes.

4.1. BC development

Regulation of signaling receptor activity is defined as any process that modulates the frequency, rate, or extent of a signaling receptor activity. Receptor activity is manifested when a molecule combines with an extracellular or intracellular messenger to initiate a change in cell activity.[38] The *INHBA* gene, upregulated and involved in this biological process (Figure 2), encodes for inhibin subunit beta A, members of the transforming growth factor β superfamily,[39] where its activation results in cell growth, proliferation, differentiation, metabolism, homeostasis, apoptosis, and carcinogenesis.[40] This gene has been previously studied in BC, where its mRNA expression was higher than in normal breast tissues.[41] Therefore, *INHBA* may serve as a prognostic indicator for BC patients.[42] In addition, it has been reported that *INHBA* signaling promotes BC metastasis by regulating *IL13Rα2* expression.[43]

LEP gene, significantly downregulated in this biological process (Figure 2), encodes for leptin, which may promote mammary tumor growth through multiple mechanisms such as modulation of the extracellular environment, downregulation of apoptosis, and/or upregulation of anti-apoptotic genes.[44] Leptin level plays a role in BC and has the potential to be developed as a diagnostic tool.[45] In premenopausal women, researchers found reduced BC risk associated with elevated leptin levels.[46] Ishikawa *et al.*[47] showed that leptin may play a role in the carcinogenesis and metastasis of BC, possibly in an autocrine manner. In a contradictory result, Niu *et al.*[45] showed that the mean serum leptin level of case groups was significantly higher than that of control groups, indicating that higher leptin may be associated with increased incidence and development of BC. Several studies have identified the associations of several polymorphisms in *LEP* with BC risk,[48,49] whereas others suggested null associations.[49,50]

Cytokine-mediated signaling pathway regulates many downstream cellular processes.[38] *FN1* gene, upregulated in this BP, has been found to be upregulated in both cancer epithelium and stroma relative to normal epithelium and stroma, respectively.[51] *FN1* encodes for a heterodimeric glycoprotein form at the cell surface and extracellular matrix that binds to interleukin[52] and is found to be involved in cell adhesion and cell metastasis.[53] The *FN1* gene was found to be a key regulator in BC development and a potential target in cancer treatment.[54] Moreover, *FN1* expression is directly regulated by microRNA-206, which has been demonstrated to be associated with metastatic cancer types, including BC.[55,56]

Response to drug is defined as any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a drug stimulus.[38] *CDK1* and *SFRP1* are associated in this BP. Specifically, *CDK1*, implicated in mammalian mitosis,[57,58] was involved in the G2/M phase arrest in many tumor types[59] as well as the initiation and development of cancer. *SFRP1* acts as a tumor suppressor gene,[60] and *SFRP1* promoters are often hypermethylated in tumor cells.[61] *SFRP1* was overexpressed in TNBC compared to other BC subtypes.[62] Knockdown of *SFRP1* increased the migration and invasion potential of tumor cells as well as reduced apoptotic events. Lack of

Figure 2. Means and standard error of the differentially expressed genes in breast cancer development, implicated in the regulation of signaling receptor activity. Cancer is represented as blue, and the control is in red.

SFRP1 protein expression is associated with poor overall survival in patients with early BC.[63]

Basal-like subtype is associated with low methylation levels of *SFRP1*, suggesting that the methylation levels of specific BC genes may potentially serve as epigenetic biomarkers and prognostic factors.[64] In BC, hypermethylation of the *SFRP1* promoter has been correlated to poor prognosis, presumably due to elevated levels of Wnt signaling,[61] indicating that the level of *SFRP1* may be associated with BC.

Negative regulation of cell proliferation and leukocyte migration are processes that control cell proliferation.[38] *INHBA* and *SFRP1* are involved in the former, while *FN*1 and *LEP* are associated with the latter.

COL10A1, *COL11A1*, *TOP2A*, *CDK1*, *MMP11*, and *S100P* were the most significantly upregulated and *ADH1B*, *SFRP1*, *DST*, *LEP*, *ADIPOQ*, and *CHRDL1* were most significantly downregulated genes in BC development. *COL10A1* was overexpressed in BC and was involved in tumor vasculature through the regulation of cell proliferation, migration, and invasion.[65-69] Protein expression of the *COL10A1* gene in the plasma could be a potential biomarker for early BC.[70] In addition, *COL11A1* was overexpressed in invasive ductal and lobular carcinomas[71] and has been reported to be a fundamental gene in late-stage BC.[72] *TOP2A* provokes tumorigenesis through chromosome instability[73,74] and DNA damage[75] and is considered a biomarker of cancer proliferation[76,77]

as it was highly overexpressed in proliferative subtypes of BC, like human epidermal growth factor receptor 2-enriched BC, luminal BC, and TNBC.[78-81] *MMP-11* was overexpressed in BC cells and is associated with poor survival rates in patients with BC.[82] It is considered a potential biomarker for early BC diagnosis and prognosis,[83] metastatic BC,[84] and immunotherapeutic strategies.[85] *S100P* was upregulated in breast and many other cancers and controlled many carcinogenic processes.[86-91]

ADH1B was underexpressed in BC cells, which may induce metabolic pathway disorders leading to carcinogenesis.[92] Moreover, overexpression of *ADH1B* correlates with a decrease in mortality in patients with gastric cancer.[93]

DST may promote BC progression and behave as a tumor suppressor in breast epithelial cells.[94] *ADIPOQ* has been reported to increase BC risk and was correlated with poor outcomes in patients with early BC.[95] *Chrdl1* has a tumor suppressor activity in BC, and its overexpression is correlated with longer disease-free and distant metastasis-free survival times.[96] Thus, *Chrdl1* upregulation might be a potential biomarker for improved outcomes in patients with BC.

4.2. BC progression

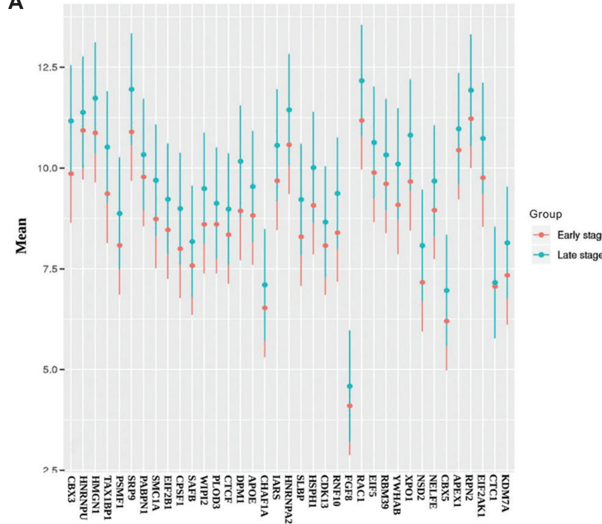
CBX3 was upregulated in the BP of cellular macromolecule biosynthesis (Figure 3A). *CBX3* silences gene expression,[97-100] and *CBX3/HP1γ* can affect transcriptional activation, alternative RNA splicing, DNA damage responses, transcription elongation, and cell growth and differentiation.[101-108] Overexpression of *CBX3/HP1γ* negatively impacts prognosis by promoting cancer development and growth in BC.[109]

The response to organic substances involving *SHARPIN* plays an important regulatory function in inflammation control.[110-113] Numerous studies have reported that *SHARPIN* is upregulated in various cancers, including BC,[114-116] and its expression correlates with poor prognosis in BC patients.[115,117,118]

Another enriched BP in BC progression is the response to endogenous stimulus, which might be linked to cancer in terms of gene expression, secretion, and cell movement.

Cell development concerns cell progression over time.[38] *RAC*, upregulated in this BP, is highly implicated in cell migration and survival by regulating cytoskeleton reorganization.[119] It has been reported to be overexpressed in BC as well as many other tumors[120] and is an indicator of cancer therapy resistance.[121-123] Furthermore, radioresistance of BC cells depends on *Rac1* signaling.[124]

Cellular protein localization harbored *YWHAZ* (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase



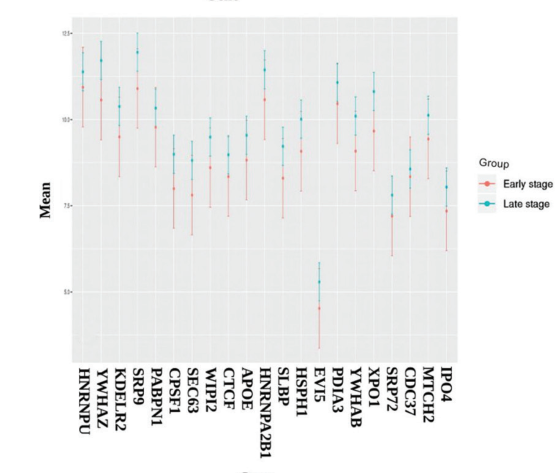


Figure 3. Means and standard error of the differentially expressed genes in breast cancer progression implicated in (A) cellular macromolecule biosynthetic process and (B) cellular protein localization. The late-stage cancer is represented in blue, and the early-stage cancer is in red.

activation protein zeta) gene that encodes for anti-apoptotic proteins.[125] This gene was overexpressed in cancer tissues (Figure 3B), and its experimental downregulation inhibited cell migration.[126] In addition, *YWHAZ* confers cancer cells apoptosis resistance and has been associated with low survival rates and drug resistance in BC patients.[127-129]

In BC progression, at least three genes were highly modulated: *DHTKD1* and *CBX3* were upregulated, and *MAP3K20* was downregulated. *DHTKD1* has been implicated in reducing cell apoptosis and increasing cell growth through mitochondrial biogenesis and function maintenance.[130] It was reported that mitochondrial dysfunction is associated with several diseases, including cancers.[131-133] Furthermore, the *DHTKD1*-encoded protein

was reported as a protein biomarker of late-stage BC.[134] *CBX3* is involved in cell growth and differentiation and DNA damage responses.[101-108] Specifically, overexpression of the *CBX3* gene is an indicator of poor prognosis in BC.[135]

The DEG discussed herein constitutes potential biomarkers for BC development and progression. Patients could be evaluated by dosing the expression of one or a set of biomarkers. Some biomarkers could be highly useful for the choice of treatment strategies. Among the robust DEGs, the *CDK* gene corresponds to known or potential drug targets. *CDK* genes, particularly *CDK4* and *CDK6*, are already established therapeutic targets in BC, with inhibitors such as palbociclib, ribociclib, and abemaciclib widely used in clinical practice. However, this study did not associate the identified DEGs identified to approved drugs, drug development pipelines, or databases.

Several genes were found to be upregulated; however, the study did not investigate whether these genes are associated with patient outcomes such as overall survival, progression-free survival, or response to specific treatments. Without linking gene expression changes to clinical endpoints, the functional and prognostic significance of these findings remains unclear. Future work should integrate survival analysis or treatment response data to identify genes with potential clinical utility.

A major limitation of this study is the absence of adjustment for critical clinical and demographic factors such as age, treatment history, hormone receptor status, and ethnicity. These variables are well-documented confounders in gene expression analyses and can significantly influence transcriptional profiles. The lack of adjustment may mask true biological signals, thereby limiting the interpretability and generalizability of our findings.

5. Conclusion

In this study, we combined many statistical methods to perform a stringent meta-analysis for BC development and progression. This approach highlighted 3,362 and 95 DEGs associated with BC development and progression, respectively. The top BP of the GO term with the associated genes were scrutinized to gain a deeper insight into BC development and progression.

Acknowledgments

None.

Funding

This study was supported by the National Centre for Scientific and Technical Research (CNRST) through the Programme de Bourses d'Excellence de Recherche (PBER).

Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

All data analyzed have been presented in the paper.

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