TPM Vol. 32, No. S1, 2025
ISSN: 1972-6325
https://www.tpmap.org/

Open Access

CHARACTERIZING THE MOLECULAR MECHANISM OF BREAST CANCER METASTASIS TO THE OVARIES

S. MRIDULA[1], AROCKIA ALEX[1], DEBALINA SEN'S[1], DR. ABIRAMI[3]
[1]DEPARTMENT OF BIOCHEMISTRY, SAVEETHA MEDICAL COLLEGE, SAVEETHA INSTITUTE OF MEDICAL AND TECHNICAL SCIENCES, SAVEETHA UNIVERSITY, CHENNAI, TAMIL NADU, INDIA.
[3]TUTOR, DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS, SREE BALAJI DENTAL COLLEGE & HOSPITAL, CHENNAI, INDIA

Abstract

Breast cancer continues to be a predominant cause of cancer-related death in women globally, with metastasis to distant organs substantially deteriorating patient prognosis. The ovaries constitute a distinctive metastatic location owing to their specific hormonal milieu, immunological interactions, and extracellular matrix composition. This work seeks to clarify the molecular pathways that regulate breast cancer metastasis to the ovaries by integrating transcriptome and proteomic analysis. Analysis of differential gene expression in ovarian cancer (GSE262869) and breast cancer (GSE31192) datasets revealed 2,393 and 1,013 differentially expressed genes (DEGs), respectively. STRING and Cytoscape analysis of 36 overlapping differentially expressed genes (DEGs) identified seven hub genes (EZH2, GZMB, NSD2, TPX2, GNAI1, SYK, and LCK), all of which were highly elevated in both malignancies. Gene ontology and KEGG pathway enrichment analysis revealed substantial participation in the PI3K/AKT/mTOR, MAPK, Wnt/β-catenin, and TGF-β signalling pathways, which govern tumour survival, invasion, and immune evasion. Epithelial-mesenchymal transition (EMT) was recognised as a primary catalyst for metastatic progression, mediated by transcription factors including Snail, Slug, Twist, and ZEB1/ZEB2. The ovarian tumour microenvironment, which is characterised by an abundance of hormones and cancer-associated fibroblasts, facilitates metastatic colonisation by promoting immune suppression and tumour development. The study emphasised the role of exosomes and microRNAs (miR-21, miR-200, and miR-10b) in influencing epithelial-mesenchymal transition (EMT) and drug resistance. A survival study using TCGA datasets revealed a significant association between the overexpression of EZH2, GZMB, NSD2, TPX2, and GNAI1 with worse patient prognosis, indicating their potential as prognostic biomarkers and therapeutic targets. Moreover, metabolic changes, including enhanced glycolysis and fatty acid oxidation, were identified in metastatic breast cancer cells, suggesting potential metabolic weaknesses for targeted treatment. This work emphasises the need for multiomics strategies to elucidate the intricacies of breast cancer metastasis to the ovaries. Identifying crucial regulatory genes and pathways provides a basis for developing tailored medicines and precision medicine tactics, eventually seeking to enhance early diagnosis, prognosis, and treatment results for patients with metastatic breast cancer.

Keywords: Breast cancer metastasis; Ovarian metastasis; Differentially expressed genes (DEGs); Transcriptomics; Proteomics; Hub genes; EZH2; GZMB; NSD2; TPX2; GNAI1; SYK; LCK; PI3K/AKT/mTOR pathway; MAPK pathway; Wnt/β-catenin signaling; TGF-β signaling; Epithelial-mesenchymal transition (EMT); Tumour microenvironment

INTRODUCTION

Breast cancer continues to be a major contributor to cancer-related deaths in women globally, and its capacity to spread to distant organs greatly deteriorates patient outcomes. The ovaries stand out as a unique and complex target among the different metastatic sites, influenced by their specific microenvironment, hormonal dynamics, and immune interactions. Metastasis represents a multifaceted and intricate biological process [1–3]. It encompasses several stages, including the detachment of cancer cells from the primary tumor, invasion of adjacent tissues, entry into the circulatory or lymphatic systems, survival while in circulation, exit from the bloodstream, and ultimately, the colonization of distant organs [4]. The molecular mechanisms that regulate the spread of breast cancer to the ovaries are shaped by

complex signaling pathways, genetic alterations, epigenetic changes, and interactions with the tumor microenvironment. Metastasis is significantly influenced by epithelial-mesenchymal transition (EMT), a crucial process that facilitates the loss of cell-cell adhesion in epithelial cancer cells while enabling them to adopt mesenchymal characteristics, thereby enhancing their invasiveness and motility [5–7]. This transition is governed by various transcription factors, including Snail, Slug, Twist, and ZEB1/ZEB2, which inhibit epithelial markers such as E-cadherin while promoting mesenchymal markers like N-cadherin and vimentin [8–10].

Besides EMT, the spread of breast cancer to the ovaries is affected by changes in critical oncogenic pathways, including the PI3K/AKT/mTOR, MAPK, Wnt/β-catenin, and TGF-β signaling cascades, which govern tumor cell survival, proliferation, invasion, and immune evasion [11,12]. The ovarian microenvironment is essential for enabling metastatic colonisation. The ovaries contain a significant amount of hormones, such as estrogen and progesterone, which can facilitate the proliferation and persistence of hormone receptor-positive breast cancer cells [13–15]. Moreover, the interplay between cancer cells and the ovarian stroma, facilitated by extracellular matrix proteins, cytokines, and chemokines, plays a significant role in the formation of metastatic lesions. Cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) present in the ovarian niche release factors that promote tumor cell adhesion, angiogenesis, and immune suppression, enabling breast cancer cells to escape immune surveillance and prosper in their new surroundings [16,17]. Recent studies underscore the significance of exosomes and microRNAs (miRNAs) in the metastatic process. Exosomes, which are tiny extracellular vesicles released by cancer cells, transport proteins, lipids, and nucleic acids that enhance intercellular communication and encourage a pro-metastatic phenotype. Specific miRNAs, including miR-21, miR-200, and miR-10b, have been associated with the regulation of EMT, drug resistance, and metastatic potential in breast cancer [18–21]. Furthermore, metabolic reprogramming plays a vital role in ovarian metastasis, as metastatic breast cancer cells demonstrate heightened glycolysis, fatty acid oxidation, and mitochondrial biogenesis to adjust to the ovarian microenvironment and support rapid proliferation. Recent advancements in high-throughput omics technologies, such as transcriptomics, proteomics, metabolomics, and single-cell sequencing, have yielded significant insights into the molecular landscape of breast cancer metastasis. Discovering distinct molecular signatures linked to ovarian metastases can contribute to the creation of innovative biomarkers for early detection and tailored therapeutic approaches [22,23]. Focusing on critical metastatic pathways via advanced treatment strategies, including small-molecule inhibitors, immunotherapies, and gene editing methods, shows potential for enhancing patient outcomes [24,25]. This study seeks to elucidate the molecular mechanisms thoroughly that govern the metastasis of breast cancer to the ovaries, highlighting essential regulatory networks that facilitate this phenomenon. A comprehensive understanding of these mechanisms will establish a basis for creating targeted therapeutic interventions and precision medicine strategies, ultimately enhancing prognosis and treatment options for patients impacted by metastatic breast cancer.

MATERIALS AND METHODS

Data collection

The raw data for the current study was obtained from the Gene Expression Omnibus (GEO) database. Criteria and constraints have been implemented to obtain the raw data for expression profile array data from a tissue or clinical sample. The accession ID GSE262869 from Gene Expression Omnibus (GEO) was utilized to obtain expression data for Ovarian cancer, while GSE31192 was employed for Breast cancer. In the study of ovarian cancer, paired tumor and normal samples from 64 patients were analyzed to identify genes that are differentially expressed. Every set of samples is linked to an individual patient, allowing for a straightforward comparison of gene expression profiles between tumorous and normal tissues. The dataset for breast cancer includes samples from 33 patients, with each patient providing two arrays: one from cancer cells and another from normal cells. The differentially expressed genes of ovarian cancer and breast cancer were analyzed for validation utilizing the GEPIA2 online tool (http://gepia2.cancer-pku.cn/#index). Following this, the DEGs that overlapped between Ovarian cancer and Breast cancer were selected based on the shared findings from the GEPIA and GEO datasets. This may reduce the impact of the variability present in the different datasets.

Data pre-processing

The Series Matrix Files for GSE262869 and GSE31192 were acquired from the GEO database to facilitate a thorough investigation. Before analysis, probe data in each dataset were converted into standard gene symbols, matching gene identities with a globally accepted nomenclature. Both datasets were normalized to guarantee homogeneity and reduce

any technical biases. The robust multi-array average (RMA) approach was used inside the R software environment (version 2.6.0) to normalize gene expression data, assuring uniformity in size and distribution across the datasets.

Identification of Differentially Expressed Genes (DEGs)

In this investigation, we employed GEO2R to analyze differentially expressed genes (DEGs) in ovarian cancer and breast cancer. This tool produced a volcano plot that displays the fold change in gene expression along the x-axis and the statistical significance (P-value) along the y-axis. To identify differentially expressed genes, we established a rigorous criterion, necessitating a p-value cutoff of <0.05 and an absolute log fold change greater than 1. In addition to this analysis, we obtained gene expression profiles for ovarian cancer and breast cancer from GEPIA2, utilizing the same criteria for identifying differentially expressed genes. Additionally, we utilized FunRich V3.1.3 to visualize the overlap and disparities in DEGs across these datasets. The Venn diagram produced by FunRich offers a precise illustration of the shared molecular targets or pathways across the datasets.

Protein-protein interaction and hub gene identification

Protein-protein interaction (PPI) analysis with STRING entails entering a list of proteins into the database to illustrate a network of anticipated connections, including both physical and functional linkages. A cumulative score over 0.08 was considered significant for the interactions, indicating the trustworthiness of the identified protein relationships. The resultant DEGs were used to create and show the PPI network using Cytoscape software (version 3.5.1; http://www.cytoscape.org). In this created PPI network, the connections between proteins were shown by edges, with widths indicating the intensity of these interactions based on the aggregated score. Hub genes in this network were found using the CytoHubba plugin inside the Cytoscape program. The research revealed hub genes, defined as nodes with a degree greater than 10, signifying their importance within the network. This integrated strategy offers a thorough means of investigating the complex network of protein interactions and pinpointing essential components in cellular activities.

mRNA expression and survival analysis of hub genes

In silico methods such as UALCAN, GEPIA, and KM Plotter were used to evaluate survival rates and gene expression relationships in ovarian and breast cancer patients. The Kaplan-Meier approach, augmented by log-rank testing, enabled the survival analysis. A connection of statistical significance was identified between gene expression levels and patient survival, according to a significance criterion of ($P < 0.05$). Data from patients with ovarian and breast cancer, obtained from The Cancer Genome Atlas, were used for expression validation. The data, expressed as transcripts per million (TPM) values, facilitated the establishment of two separate groups. The GEPIA database was used to illustrate these categories, categorizing patients with TPM levels below the upper quartile into the low/medium expression group and those with TPM values above the upper quartile into the high expression group.

Gene ontology and pathway enrichment analysis

The database for annotation, visualization, and integrated discovery (DAVID, https://david.ncifcrf.gov/tools.jsp) was used to analyze the GO and KEGG pathway enrichment of differentially expressed genes (DEGs). A P-value of less than 0.05 was established as the threshold. The research used KEGG pathway analysis to discover pathways highly enriched about the differentially expressed genes (DEGs). Pathway crosstalk analysis was conducted using specific criteria: a Benjamini-Hochberg adjusted p-value of less than 0.05 and both a Jaccard coefficient and an overlap coefficient surpassing 0.5, which were considered statistically significant. This comprehensive analysis of the DEGs inside certain pathways demonstrates their potential role in critical biological processes and regulatory networks.

TPM Vol. 32, No. S1, 2025
ISSN: 1972-6325
https://www.tpmap.org/

Open Access

RESULT

Identification of DEGs in Ovarian cancer and Breast cancer

From the Ovarian cancer cancer dataset GSE262869, 2393 DEGs were identified (Figure 1) and from the Breast cancer dataset GSE31192, 1013 DEGs were detected (Figure 2). The identification was performed through GEO2R analysis utilizing the Limma package, with stringent selection criteria of an adjusted p-value of <0.05 and a log fold change of >1. Subsequently, this process facilitated the generation of volcano plots for each dataset.

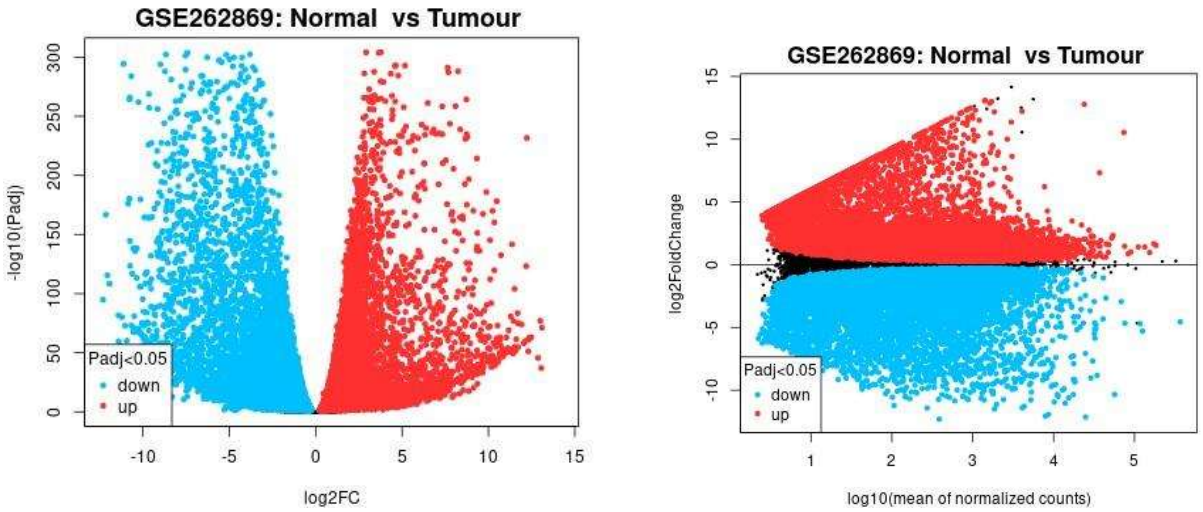


Figure 1

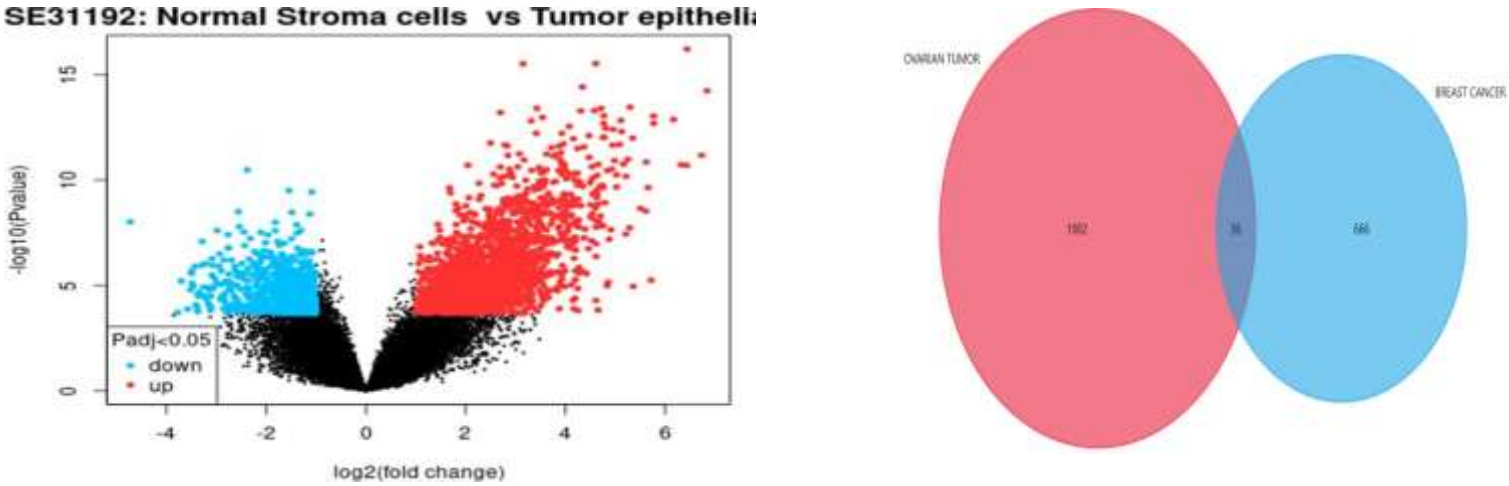


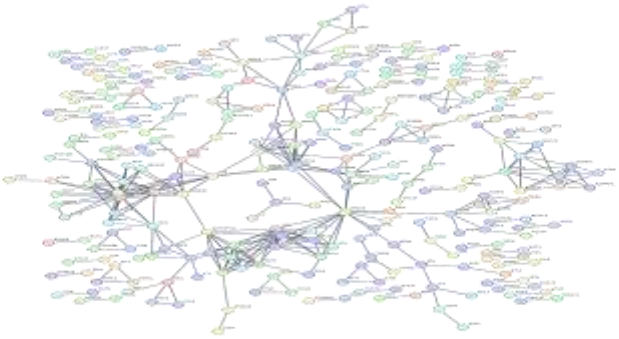
Figure 2

PPI network construction and hub gene identification

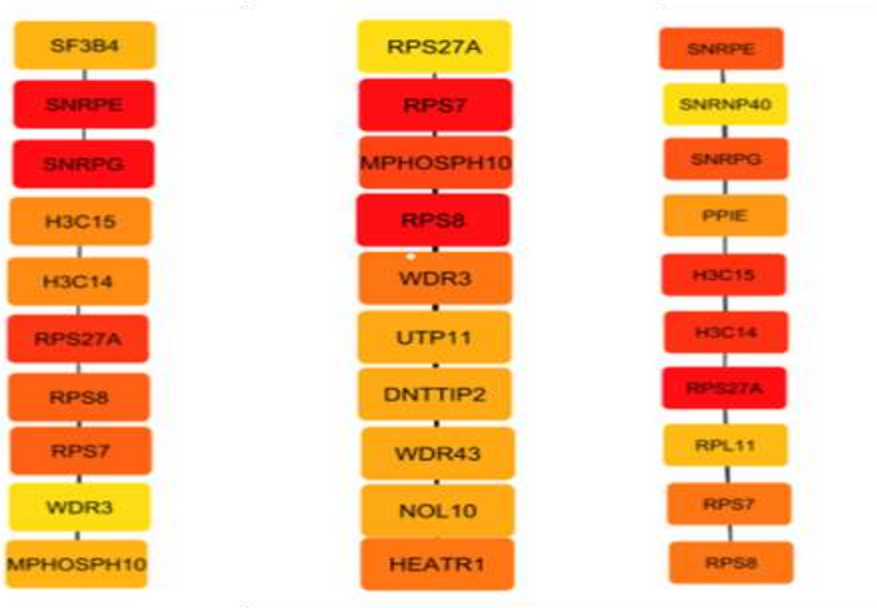
A protein-protein interaction (PPI) network was constructed for proteins generated by 36 overlapping differentially expressed genes (DEGs) by STRING analysis (Figure 2A). Nineteen of the thirty-six DEGs were shown to be linked, as demonstrated by Cytoscape visualization. Furthermore, seven hub genes (EZH2, GZMB, NSD2, TPX2, GNAI1,

SYK, LCK) were found by methodologies including MCC, closeness, and degree centrality (Figure 2B). All these hub genes exhibited up-regulation in the overlapping DEGs, indicating their potential significance in the progression of both ovarian and breast cancer.

A



B



RPS8	SNRPG	RPS27A
RPS7	SNRPE	H3C14
MPHOSPH10	RPS27A	H3C15
WDR3	RPS8	SNRPG
HEATR1	RPS7	SNRPE
DNTTIP2	H3C14	RPS8
UTP11	H3C15	RPS7
NOL10	MPHOSPH10	PPIE
WDR43	SF3B4	RPL11
RPS27A	WDR3	SNRNP40

FIGURE 2: Protein and protein interaction network
Interaction network of 36 DEGs (A). Cytoscape cytohubba pluggin identified the top 10 hub genes by MCC, degree and Closeness (B). Cytohubba methods rank hub genes

Gene ontology and KEGG pathway analysis of DEGs

The gene ontology study for ovarian and breast cancer elucidates several biological processes, cellular components, and molecular activities that are closely associated with the prognosis of ovarian metastases in breast cancer patients. The concentration of genes associated with cellular matrix architecture and cell-substrate adhesion indicates a crucial function in the detachment and invasion of cancer cells, enabling their spread from the original tumour to the lungs. Epithelial cell proliferation signifies accelerated tumour development, a characteristic of aggressive malignancies with increased metastatic potential. Neutrophil activation in the immune response may influence a milieu that either facilitates or obstructs metastatic dissemination, contingent upon the equilibrium of pro- and anti-tumour elements. From a biological component standpoint, the collagen-rich extracellular matrix and intercellular junctions are crucial for preserving tissue architecture; their disturbance may facilitate metastatic escape. Membrane rafts and microdomains, in conjunction with the basolateral plasma membrane and desmosome structures, are involved in signal transduction and cellular adhesion, both essential for the metastatic process. Molecular activities, including integrin binding, cadherin binding, and growth factor binding, illustrate the intricate connections between cells and their surroundings, affecting cell migration and survival during metastasis. Peptidase regulators and inhibitors, by altering proteolytic activity, contribute to extracellular matrix remodelling, an essential factor for metastatic development. The pathway enrichment analysis of differentially expressed genes in ovarian and breast cancer highlights the intricacies of ovarian metastasis in breast cancer. The ECM-receptor interaction and focal adhesion pathways are essential for cell motility and adhesion, enabling the detachment and dissemination of cancer cells. The cell cycle and DNA replication pathways signify enhanced cellular proliferation, a characteristic of metastatic cells. Glycolysis exemplifies the metabolic reprogramming of neoplastic cells, facilitating their survival and proliferation. The relaxin signalling pathway influences the tumour microenvironment, facilitating metastasis. The complement and coagulation cascades may augment the viability of circulating tumour cells, while phenylalanine metabolism plays a role in manufacturing chemicals that facilitate tumour development and metastasis. These pathways jointly elucidate possible causes and treatment targets for the prevention of ovarian metastases in breast cancer patients (Figure 3 and 4).

Figure 3

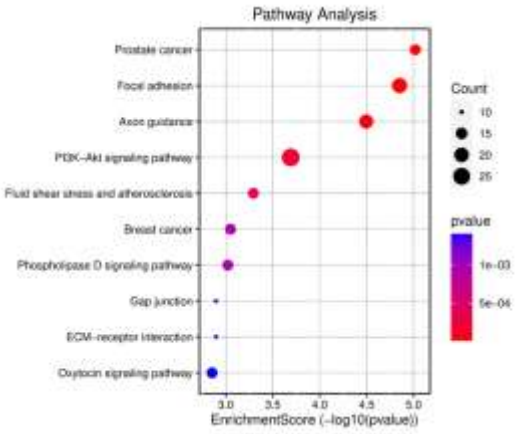


Figure 4

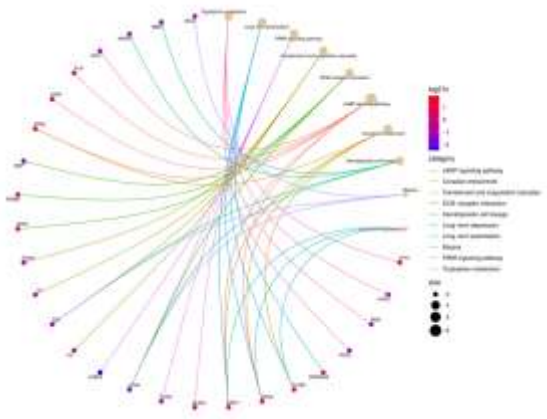
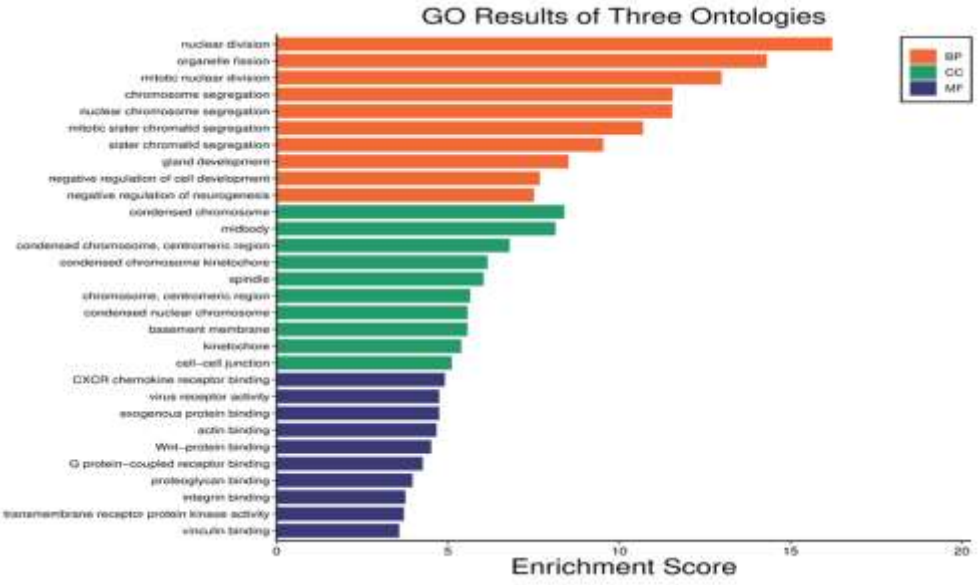


Figure 6

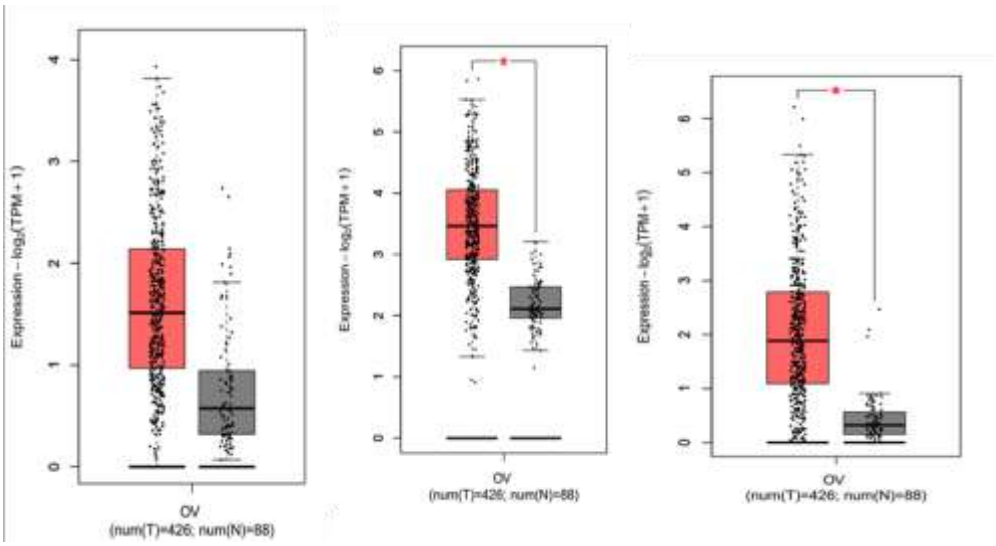


Verification and survival analysis of hub genes in Ovarian cancer and Breast cancer

A comparative mRNA expression study of hub genes was conducted for ovarian cancer and breast cancer using the GEPIA platform. The research demonstrated a significant overexpression of all seven hub genes in both cancer types (Figure 7). This increase is notably noteworthy as it may influence the prognosis of ovarian metastases in individuals with breast cancer. The persistent upregulation of these genes in both ovarian and breast cancer may elucidate the molecular processes that enable the dissemination of cancer cells to the ovaries, the most common location of distant metastasis from breast cancer. The relationship between the expression levels of pivotal hub genes and the stages of patients' cancer was examined using the UALCAN platform. The detected overexpression of hub genes in grade 2 and grade 3 ovarian cancer, along with stage 2 and stage 3 breast cancer, indicates a link with the advancement of these malignancies. This gene expression pattern signifies an increased potential for tumour invasion and metastasis,

especially to the ovaries, as the illness progresses. The continual rise across various grades and stages suggests that these genes may be essential to the mechanisms underlying the metastatic process, potentially acting as biomarkers for aggressive disease and as targets for therapeutic strategies aimed at reducing ovarian metastasis in breast cancer patients. A survival study of ovarian and breast cancer patients in TCGA was conducted based on hub genes using UALCAN (Figures 8 and 9). In ovarian and breast cancer, survival studies indicate that elevated gene expression of EZH2, GZMB, NSD2, TPX2, and GNAI1 strongly correlates with patient outcomes, as shown by p-values below 0.05. The very low p-values for GZMB (0.0033) and NSD2 (0.011) indicate a robust correlation with survival, underscoring their potential as essential biomarkers for rapid disease advancement. EZH2, TPX2, and GNAI1 exhibit significant relationships, with p-values of 0.031, 0.039, and 0.021, respectively, underscoring their importance in patient prognosis. The expression of TPX2, with a p-value of 0.039, nears statistical significance, indicating a potential but inconclusive impact on patient survival outcomes. This suggests that while TPX2 may have a role in prognosis, its influence is not as clearly established as that of genes with p-values significantly below 0.05. GNAI1, with a p-value of 0.021, did not demonstrate a statistically significant connection, indicating that its expression may not be a dependable prognostic marker for survival in these malignancies. These data together highlight the significance of these genes in the pathogenesis of ovarian and breast cancer, with GZMB and NSD2 emerging as especially intriguing candidates for future investigation and possible therapeutic intervention. This study's findings may enable customized medical procedures by tailoring therapies to each patient's genetic profile.

FIGURE 7: The mRNA expression of hub genes in breast cancer. The expression levels of these hub genes were compared between cancer patient samples (in red) and normal samples (in grey).



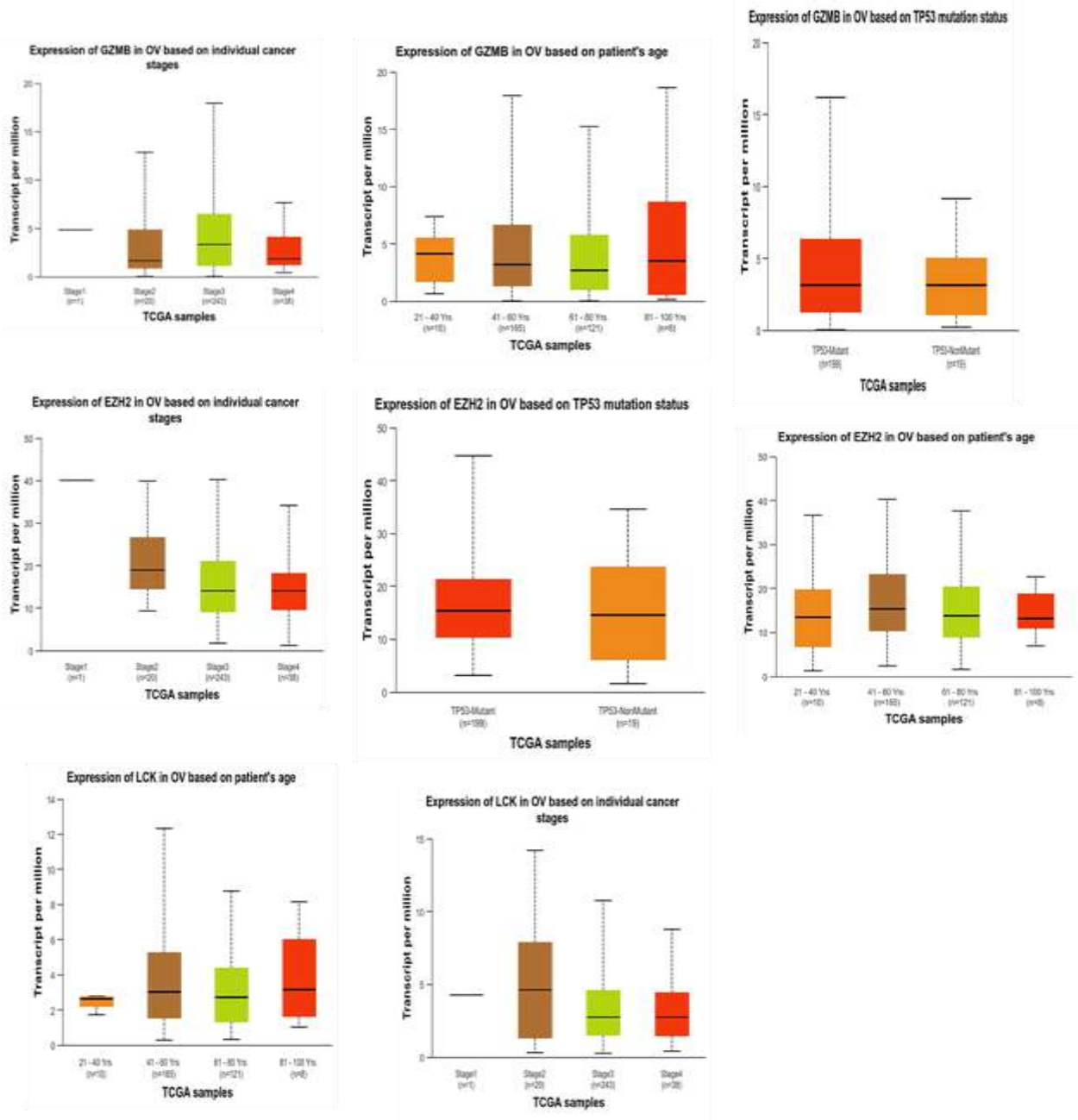


FIGURE 8: Stages of HNSCC and LSCC with relevant to expression of hub genes (EZH2, GZMB, NSD2, TPX2, and GNAI1)

TPM Vol. 32, No. S1, 2025
ISSN: 1972-6325
https://www.tpmap.org/

Open Access

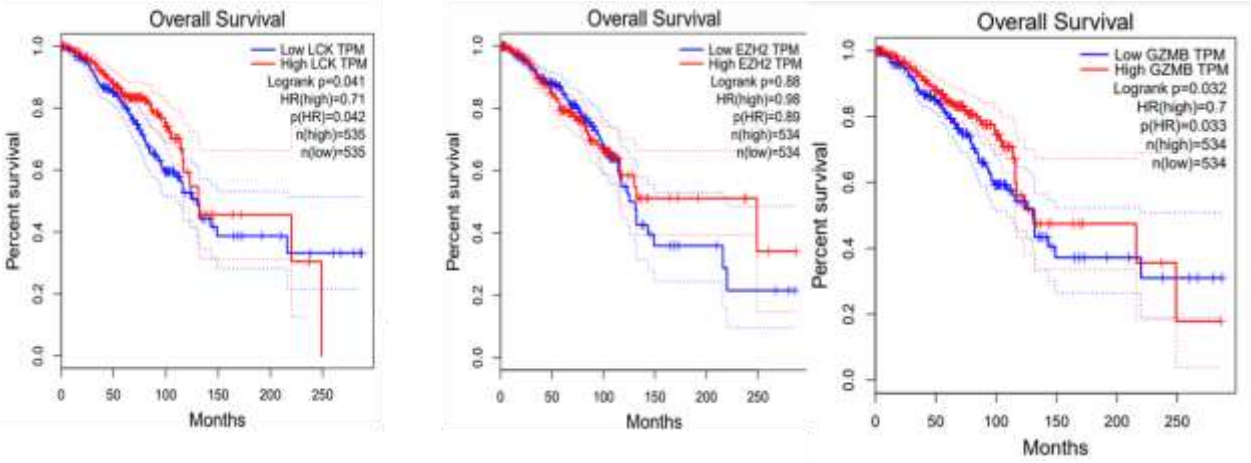


FIGURE 9: Overall survival analysis of the hub genes

Gene symbol	Gene title	Log2(fold change)	Chromosome number
CXCL11	C-X-C motif chemokine ligand 11	4.052	*chr4:76,033,682-76,041,415*
LRRC15	leucine rich repeat containing 15	4.004	*chr3:194,355,249-194,369,743*
COL10A1	collagen type X alpha 1 chain	3.717	*chr6:116,118,909-116,217,144*
CXCL10	C-X-C motif chemokine ligand 10	3.666	*chr4:76,021,118-76,023,497*
NEK2	NIMA related kinase 2	3.559	*chr1:211,658,256-211,675,630*
S100P	S100 calcium binding protein P	3.464	*chr4:6,693,878-6,697,170*
BMPR1B	bone morphogenetic protein receptor type 1B	3.403	*chr4:94,757,955-95,158,450*
ADAMDEC1	ADAM like decysin 1	3.347	*chr8:24,384,285-24,406,013*
BMPR1B	bone morphogenetic protein receptor type 1B	3.218	*chr4:94,757,955-95,158,450*
CXCL9	C-X-C motif chemokine ligand 9	3.186	*chr4:76,001,275-76,007,509*

Gene Symbol	Gene title	Log2(fold change)	Chromosome number
LINC02593	long intergenic non-protein coding RNA 2593	-6.942	*chr1:914,887-925,604*
RNF223	ring finger protein 223	5.944	*chr1:1,070,967-1,074,306*
LINC01342	long intergenic non-protein coding RNA 1342	5.777	*chr1:1,137,016-1,144,057*
MIR429	microRNA 429	6.084	*chr1:1,169,005-1,169,087*
TTLL10	tubulin tyrosine ligase like 10	5.102	*chr1:1,173,880-1,197,936*
TNFRSF18	TNF receptor superfamily member 18	8.71	*chr1:1,203,508-1,206,592*
MXRA8	matrix remodelling associated 8	-5.83	*chr1:1,352,689-1,363,541*
VWA1	von Willebrand factor A domain containing 1	5.323	*chr1:1,434,861-1,442,882*
PRDM16-DT	PRDM16 divergent transcript	5.553	*chr1:3,055,438-3,070,871*
ARHGEF16	Rho guanine nucleotide exchange factor 16	9.158	*chr1:3,454,665-3,481,113*

DISCUSSION

This study's results provide essential insights into the molecular underpinnings of breast cancer metastasis to the ovaries. The discovery of differentially expressed genes (DEGs) common to breast and ovarian malignancies highlights the same biological mechanisms that promote tumor growth and metastatic colonization [26,27]. Our findings underscore critical oncogenic pathways, including PI3K/AKT/mTOR, MAPK, Wnt/β-catenin, and TGF-β, that govern tumor cell survival, proliferation, invasion, and immune evasion. Epithelial-mesenchymal transition (EMT) is a significant driver of metastasis, with transcription factors including Snail, Slug, Twist, and ZEB1/ZEB2 crucial for facilitating cellular plasticity and increased motility [28,29]. The ovarian microenvironment is crucial for metastatic development, offering a distinctive niche rich in hormones, extracellular matrix elements, and immunological modulators. Our results indicate that hormone receptor-positive breast cancer cells use the estrogen- and progesterone-abundant environment of the ovaries to maintain their development and viability. Moreover, interactions with cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) promote immune evasion and angiogenesis, thereby enhancing metastatic colonization. Exosomal signaling and microRNA (miRNA) modulation have emerged as pivotal factors in the metastatic cascade, with particular miRNAs, including miR-21, miR-200, and miR-10b, affecting epithelial-mesenchymal transition (EMT), treatment resistance, and metastatic capability.

Metabolic reprogramming was recognized as a vital element in metastatic adaptability, characterized by increased glycolysis, fatty acid oxidation, and mitochondrial biogenesis in breast cancer cells that metastasized to the ovaries. This metabolic alteration allows cancer cells to flourish in a nutrient-scarce microenvironment, facilitating fast growth and survival under hypoxic settings. These results highlight potential metabolic targets for therapeutic intervention.

The findings from protein-protein interaction (PPI) network analysis further underscore the importance of essential regulatory genes in metastatic advancement. The discovery of hub genes such as EZH2, GZMB, NSD2, TPX2, GNAI1, SYK, and LCK indicates their potential as biomarkers for breast cancer metastasis to the ovaries[30–32]. EZH2, a prominent epigenetic regulator, has been associated with enhancing tumor aggressiveness via chromatin remodeling and the suppression of tumor suppressor pathways. Likewise, GZMB and NSD2 exhibited a substantial correlation with worse patient prognosis, highlighting their probable involvement in metastatic disease advancement [33–36]. The survival study indicates that the overexpression of these hub genes correlates with worse survival rates in patients with breast and ovarian cancer, highlighting their clinical significance.

Gene ontology (GO) and KEGG pathway enrichment analysis provide further mechanistic insights into the basic mechanisms regulating metastasis. Pathways including ECM-receptor interaction, focal adhesion, and cell cycle control were significantly enriched, underscoring their vital involvement in metastatic spread. The participation of complement and coagulation cascades indicates that circulating tumor cells may use these pathways to improve their survival in the circulation, hence aiding effective metastatic colonization [37–43].

The incorporation of high-throughput omics technologies, including transcriptomics, proteomics, and metabolomics, has greatly enhanced our comprehension of metastatic breast cancer. Our research underscores the need to use multiomics methodologies to discover new treatment targets and biomarkers for early identification. Utilizing these findings, further research may concentrate on formulating focused therapy options, such as small-molecule inhibitors, immunotherapies, and gene-editing technologies, to obstruct critical metastatic pathways [44–46].

Notwithstanding these encouraging results, many restrictions must be recognized. Initially, our research mostly depends on publicly accessible datasets, which may include unpredictability stemming from discrepancies in sample processing and experimental circumstances. Moreover, functional confirmation of the identified hub genes and pathways in both in vitro and in vivo models is essential to ascertain their involvement in ovarian metastasis. Future research should prioritize experimental validation using patient-derived xenograft models and single-cell sequencing to enhance understanding of tumor heterogeneity and the metastatic potential of breast cancer cells [47,48] .

In conclusion, our work offers an extensive molecular analysis of breast cancer metastasis to the ovaries, pinpointing critical regulatory genes, signaling pathways, and metabolic changes that facilitate this process. This study's findings facilitate the creation of focused therapies and individualized treatment methods to enhance patient outcomes in metastatic breast cancer.

Numerous studies have shown that epithelial-mesenchymal transition (EMT) is a pivotal mechanism in breast cancer metastasis [49]. A previous study showed that EMT transcription factors, including Snail, Slug, and Twist, augment the invasiveness of breast cancer cells. Furthermore, breast cancer cells undergoing epithelial-mesenchymal transition (EMT) have enhanced resistance to apoptosis and elevated metastatic potential, perhaps explaining the augmented

expression of EZH2 and NSD2 seen in our work. Recent investigations have corroborated the importance of tumor microenvironment interactions in ovarian metastasis. The ovarian stroma is crucial in promoting metastasis via the secretion of pro-inflammatory cytokines and extracellular matrix components that facilitate tumor adherence and immune evasion [50–52].
Our research corroborates the results who examined the function of tumor-associated macrophages (TAMs) in ovarian metastasis and identified their contribution to a pre-metastatic niche that promotes the survival of disseminated breast cancer cells. Furthermore, prior research using high-throughput omics technology has shown significant metabolic alterations in metastatic breast cancer cells. The process by which cancer cells engage in metabolic reprogramming to facilitate their proliferation in disparate microenvironments, perhaps accounting for the substantial enrichment of metabolic pathways, such as glycolysis and fatty acid oxidation, seen in our research.
The survival study reinforces the clinical importance of the discovered hub genes, especially GZMB and NSD2, which exhibited substantial predictive value. The same data, indicating that GZMB overexpression correlates with reduced survival in ovarian cancer patients, suggesting its potential as a biomarker for metastatic progression. The increase of TPX2, a gene implicated in mitotic progression, aligns with prior research associating it with aggressive breast cancer subtypes and worse patient prognosis.

CONCLUSION

This work offers an in-depth examination of the molecular processes involved in breast cancer metastasis to the ovaries, pinpointing essential regulatory genes and pathways that facilitate tumour growth and metastatic colonisation. By employing an integrative methodology that encompasses differential gene expression analysis, protein-protein interaction networks, and survival analysis, we identified seven hub genes (EZH2, GZMB, NSD2, TPX2, GNAI1, SYK, and LCK) that demonstrate significant upregulation in both ovarian and breast cancer, indicating their pivotal role in metastatic progression. The PI3K/AKT/mTOR, MAPK, Wnt/β-catenin, and TGF-β signalling pathways were significantly enriched, underscoring their roles in tumour cell survival, proliferation, invasion, and immune evasion. Our results underscore the pivotal role of epithelial-mesenchymal transition (EMT) in enabling the metastasis of breast cancer cells to the ovaries, with essential transcription factors including Snail, Slug, Twist, and ZEB1/ZEB2 promoting cellular plasticity and motility. Moreover, the ovarian milieu, saturated with hormones and stromal elements, creates a favourable habitat for metastatic colonisation, which facilitates tumour proliferation and immune evasion. The role of exosomal signalling and microRNA regulation, namely miR-21, miR-200, and miR-10b, highlights the complexity of breast cancer metastasis to the ovaries. The survival study revealed a significant association between the overexpression of several hub genes (EZH2, GZMB, NSD2, TPX2, and GNAI1) with worse prognosis, indicating their potential as prognostic biomarkers and therapeutic targets. The metabolic modifications identified in metastatic breast cancer cells, such as increased glycolysis and fatty acid oxidation, underscore new opportunities for therapeutic intervention. Our research highlights the need to combine transcriptomic, proteomic, and pathway investigations to elucidate the complexities of metastatic progression. This work elucidates the molecular fingerprints linked to breast cancer metastases to the ovaries, establishing a platform for targeted therapeutics and precision medicine initiatives. Subsequent studies need to concentrate on corroborating these results in more extensive patient populations and investigating the therapeutic potential of inhibiting critical metastatic pathways. A comprehensive knowledge of these molecular pathways would facilitate early identification, refine prognostic evaluations, and enable innovative treatment strategies to reduce breast cancer metastasis and better patient outcomes.

REFERENCE

1. Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast cancer-epidemiology, risk factors, classification, prognostic markers, and current treatment strategies-an updated review. Cancers (Basel). 2021;13: 4287. doi:10.3390/cancers13174287

2. Menon G, Alkabban FM, Ferguson T. Breast cancer. StatPearls. Treasure Island (FL): StatPearls Publishing; 2025. Available: https://www.ncbi.nlm.nih.gov/books/NBK482286/

3. Worzfeld T, Pogge von Strandmann E, Huber M, Adhikary T, Wagner U, Reinartz S, et al. The unique molecular and cellular microenvironment of ovarian cancer. Front Oncol. 2017;7: 24. doi:10.3389/fonc.2017.00024

4. van Zijl F, Krupitza G, Mikulits W. Initial steps of metastasis: cell invasion and endothelial transmigration. Mutat Res. 2011;728: 23–34. doi:10.1016/j.mrrev.2011.05.002

5. Mathur R, Jha NK, Saini G, Jha SK, Shukla SP, Filipejová Z, et al. Epigenetic factors in breast cancer therapy. Front Genet. 2022;13: 886487. doi:10.3389/fgene.2022.886487

6. Yuan J, Yang L, Zhang H, Beeraka NM, Zhang D, Wang Q, et al. Decoding tumor microenvironment: EMT modulation in breast cancer metastasis and therapeutic resistance, and implications of novel immune checkpoint blockers. Biomed Pharmacother. 2024;181: 117714. doi:10.1016/j.biopha.2024.117714

7. Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, et al. Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes Dis. 2018;5: 77–106. doi:10.1016/j.gendis.2018.05.001

8. Pawlicka M, Gumbarewicz E, Błaszczak E, Stepulak A. Transcription factors and markers related to epithelial-mesenchymal transition and their role in resistance to therapies in head and neck cancers. Cancers (Basel). 2024;16. doi:10.3390/cancers16071354

9. Casas E, Kim J, Bendesky A, Ohno-Machado L, Wolfe CJ, Yang J. Snail2 is an essential mediator of Twist1-induced epithelial mesenchymal transition and metastasis. Cancer Res. 2011;71: 245–254. doi:10.1158/0008-5472.CAN-10-2330

10. Kaufhold S, Bonavida B. Central role of Snail1 in the regulation of EMT and resistance in cancer: a target for therapeutic intervention. J Exp Clin Cancer Res. 2014;33: 62. doi:10.1186/s13046-014-0062-0

11. Glaviano A, Foo ASC, Lam HY, Yap KCH, Jacot W, Jones RH, et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. Mol Cancer. 2023;22: 138. doi:10.1186/s12943-023-01827-6

12. Fu D, Hu Z, Xu X, Dai X, Liu Z. Key signal transduction pathways and crosstalk in cancer: Biological and therapeutic opportunities. Transl Oncol. 2022;26: 101510. doi:10.1016/j.tranon.2022.101510

13. Kozieł MJ, Piastowska-Ciesielska AW. Estrogens, estrogen receptors and tumor microenvironment in ovarian cancer. Int J Mol Sci. 2023;24. doi:10.3390/ijms241914673

14. Ho S-M. Estrogen, progesterone and epithelial ovarian cancer. Reprod Biol Endocrinol. 2003;1: 73. doi:10.1186/1477-7827-1-73

15. Chen B, Ye P, Chen Y, Liu T, Cha J-H, Yan X, et al. Involvement of the estrogen and progesterone axis in cancer stemness: Elucidating molecular mechanisms and clinical significance. Front Oncol. 2020;10: 1657. doi:10.3389/fonc.2020.01657

16. Prakash J, Shaked Y. The interplay between extracellular matrix remodeling and cancer therapeutics. Cancer Discov. 2024;14: 1375–1388. doi:10.1158/2159-8290.CD-24-0002

17. Zhang W, Wang J, Liu C, Li Y, Sun C, Wu J, et al. Crosstalk and plasticity driving between cancer-associated fibroblasts and tumor microenvironment: significance of breast cancer metastasis. J Transl Med. 2023;21: 827. doi:10.1186/s12967-023-04714-2

18. Syed SN, Brüne B. Exosomal and non-exosomal MicroRNAs: New kids on the block for cancer therapy. Int J Mol Sci. 2022;23: 4493. doi:10.3390/ijms23094493

19. Santos P, Almeida F. Role of exosomal miRNAs and the tumor microenvironment in drug resistance. Cells. 2020;9: 1450. doi:10.3390/cells9061450

20. Nail HM, Chiu C-C, Leung C-H, Ahmed MMM, Wang H-MD. Exosomal miRNA-mediated intercellular communications and immunomodulatory effects in tumor microenvironments. J Biomed Sci. 2023;30: 69. doi:10.1186/s12929-023-00964-w

21. Zhang L, Yu D. Exosomes in cancer development, metastasis, and immunity. Biochim Biophys Acta Rev Cancer. 2019;1871: 455–468. doi:10.1016/j.bbcan.2019.04.004

22. Rossi C, Cicalini I, Cufaro MC, Consalvo A, Upadhyaya P, Sala G, et al. Breast cancer in the era of integrating "Omics" approaches. Oncogenesis. 2022;11: 17. doi:10.1038/s41389-022-00393-8

23. Zhu Z, Jiang L, Ding X. Advancing breast cancer heterogeneity analysis: Insights from genomics, transcriptomics and proteomics at bulk and single-cell levels. Cancers (Basel). 2023;15: 4164. doi:10.3390/cancers15164164

24. Wu X, Feng N, Wang C, Jiang H, Guo Z. Small molecule inhibitors as adjuvants in cancer immunotherapy: enhancing efficacy and overcoming resistance. Front Immunol. 2024;15: 1444452. doi:10.3389/fimmu.2024.1444452

25. Garg P, Singhal G, Pareek S, Kulkarni P, Horne D, Nath A, et al. Unveiling the potential of gene editing techniques in revolutionizing Cancer treatment: A comprehensive overview. Biochim Biophys Acta Rev Cancer. 2025;1880: 189233. doi:10.1016/j.bbcan.2024.189233

26. Kirschmann DA, Seftor EA, Nieva DR, Mariano EA, Hendrix MJ. Differentially expressed genes associated with the metastatic phenotype in breast cancer. Breast Cancer Res Treat. 1999;55: 127–136. doi:10.1023/a:1006188129423

27. Martin TA, Ye L, Sanders AJ, Lane J, Jiang WG. Cancer Invasion and Metastasis: Molecular and Cellular Perspective. Landes Bioscience; 2013. Available: https://www.ncbi.nlm.nih.gov/books/NBK164700/

28. Abasifarid A, Dorostkar R, Ghalavand M. miR-122 shows potential as a biomarker and therapeutic target in NSCLC by inhibiting WNT/β-catenin and PI3K/AKT pathways and key transcription factors linked to EMT and metastasis. Heliyon. 2025; e42961. doi:10.1016/j.heliyon.2025.e42961

29. Li Q, Geng S, Luo H, Wang W, Mo Y-Q, Luo Q, et al. Signaling pathways involved in colorectal cancer: pathogenesis and targeted therapy. Signal Transduct Target Ther. 2024;9: 266. doi:10.1038/s41392-024-01953-7

30. Hozhabri H, Ghasemi Dehkohneh RS, Razavi SM, Razavi SM, Salarian F, Rasouli A, et al. Comparative analysis of protein-protein interaction networks in metastatic breast cancer. PLoS One. 2022;17: e0260584. doi:10.1371/journal.pone.0260584

31. Rezaei-Tavirani M, Rezaei-Taviran S, Mansouri M, Rostami-Nejad M, Rezaei-Tavirani M. Protein-protein interaction network analysis for a biomarker panel related to human esophageal adenocarcinoma. Asian Pac J Cancer Prev. 2017;18: 3357–3363. doi:10.22034/APJCP.2017.18.12.3357

32. Elbashir MK, Mohammed M, Mwambi H, Omolo B. Identification of hub genes associated with breast cancer using integrated gene expression data with protein-protein interaction network. Appl Sci (Basel). 2023;13: 2403. doi:10.3390/app13042403

33. Sun S, Yu F, Xu D, Zheng H, Li M. EZH2, a prominent orchestrator of genetic and epigenetic regulation of

solid tumor microenvironment and immunotherapy. Biochim Biophys Acta Rev Cancer. 2022;1877: 188700. doi:10.1016/j.bbcan.2022.188700

34. Gan L, Yang Y, Li Q, Feng Y, Liu T, Guo W. Epigenetic regulation of cancer progression by EZH2: from biological insights to therapeutic potential. Biomark Res. 2018;6: 10. doi:10.1186/s40364-018-0122-2

35. Duan R, Du W, Guo W. EZH2: a novel target for cancer treatment. J Hematol Oncol. 2020;13: 104. doi:10.1186/s13045-020-00937-8

36. Parkes A, Warneke CL, Clifton K, Al-Awadhi A, Oke O, Pestana RC, et al. Prognostic factors in patients with metastatic breast cancer with bone-only metastases. Oncologist. 2018;23: 1282–1288. doi:10.1634/theoncologist.2018-0085

37. Zhang W, Liu J, Zhou Y 'nan, Liu S, Wu J, Jiang H, et al. Signaling pathways and regulatory networks in quail skeletal muscle development: insights from whole transcriptome sequencing. Poult Sci. 2024;103: 103603. doi:10.1016/j.psj.2024.103603

38. Danishuddin, Haque MA, Malik MZ, Arya R, Singh P, Lee J-S, et al. Unveiling the mechanisms underlying the immunotherapeutic potential of gene-miRNA and drugs in head and neck cancer. Pharmaceuticals (Basel). 2024;17: 921. doi:10.3390/ph17070921

39. Yin H, Wang S, Zhang Y-H, Cai Y-D, Liu H. Analysis of important gene ontology terms and biological pathways related to pancreatic cancer. Biomed Res Int. 2016;2016: 7861274. doi:10.1155/2016/7861274

40. Elgundi Z, Papanicolaou M, Major G, Cox TR, Melrose J, Whitelock JM, et al. Cancer metastasis: The role of the extracellular matrix and the heparan sulfate proteoglycan perlecan. Front Oncol. 2019;9: 1482. doi:10.3389/fonc.2019.01482

41. Nersisyan S, Novosad V, Engibaryan N, Ushkaryov Y, Nikulin S, Tonevitsky A. ECM-receptor regulatory network and its prognostic role in colorectal cancer. Front Genet. 2021;12: 782699. doi:10.3389/fgene.2021.782699

42. Dasgupta A, Lim AR, Ghajar CM. Circulating and disseminated tumor cells: harbingers or initiators of metastasis? Mol Oncol. 2017;11: 40–61. doi:10.1002/1878-0261.12022

43. Dianat-Moghadam H, Azizi M, Eslami-S Z, Cortés-Hernández LE, Heidarifard M, Nouri M, et al. The role of circulating Tumor Cells in the metastatic cascade: Biology, technical challenges, and clinical relevance. Cancers (Basel). 2020;12: 867. doi:10.3390/cancers12040867

44. Zhang C, Li N, Zhang P, Jiang Z, Cheng Y, Li H, et al. Advancing precision and personalized breast cancer treatment through multi-omics technologies. Am J Cancer Res. 2024;14: 5614–5627. doi:10.62347/MWNZ5609

45. Orsini A, Diquigiovanni C, Bonora E. Omics technologies improving breast cancer research and diagnostics. Int J Mol Sci. 2023;24. doi:10.3390/ijms241612690

46. Luo Y, Li Y, Fang M, Wang S, Shao L, Zou R, et al. Multi-omics synergy in oncology: Unraveling the complex interplay of radiomic, genoproteomic, and pathological data. Intelligent Oncology. 2025;1: 17–30. doi:10.1016/j.intonc.2024.10.003

47. Zhao Y, Pi J, Liu L, Yan W, Ma S, Hong L. Identification of the hub genes associated with the prognosis of ovarian cancer patients via integrated bioinformatics analysis and experimental validation. Cancer Manag Res. 2021;13: 707–721. doi:10.2147/CMAR.S282529

48. Wang Z, Wu D, Xia Y, Yang B, Xu T. Identification of hub genes and compounds controlling ovarian cancer stem cell characteristics via stemness indices analysis. Ann Transl Med. 2021;9: 379. doi:10.21037/atm-20-3621

49. Wang Y, Zhou BP. Epithelial-mesenchymal transition in breast cancer progression and metastasis. Chin J Cancer. 2011;30: 603–611. doi:10.5732/cjc.011.10226

50. Debnath P, Huirem RS, Dutta P, Palchaudhuri S. Epithelial-mesenchymal transition and its transcription factors. Biosci Rep. 2022;42. doi:10.1042/BSR20211754

51. Du B, Shim JS. Targeting epithelial-mesenchymal transition (EMT) to overcome drug resistance in cancer. Molecules. 2016;21: 965. doi:10.3390/molecules21070965

52. Garlisi B, Lauks S, Aitken C, Ogilvie LM, Lockington C, Petrik D, et al. The complex tumor microenvironment in ovarian cancer: Therapeutic challenges and opportunities. Curr Oncol. 2024;31: 3826–3844. doi:10.3390/curroncol31070283