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COMPREHENSIVE GENE EXPRESSION PROFILING AND FUNCTIONAL ANALYSIS OF PROSTATE CANCER AND ITS METASTATIC PROGRESSION TO BONE USING MICROARRAY DATA

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Abstract

Prostate cancer (PCa) continues to be a primary cause of cancer-related death in men, with bone metastases being the most prevalent and fatal consequence of advanced illness. This work seeks to clarify the molecular pathways driving prostate cancer growth and its metastatic spread to bone by extensive gene expression profiling and functional analysis of microarray datasets. Differentially expressed genes (DEGs) were found on the GSE69223 (prostate cancer) and GSE14359 (osteosarcoma) datasets using GEO2R and rigorous statistical criteria. A total of 1326 differentially expressed genes (DEGs) were studied in prostate cancer (PCa) and 375 DEGs in osteosarcoma, and the shared genes were used to create a protein-protein interaction (PPI) network with STRING and Cytoscape. Hub genes, including TIMP3, CAV1, ECM1, IGF1, FGF2, and EGF, were recognized as essential contributors to tumor growth. Functional enrichment using Gene Ontology (GO) and KEGG studies indicated severe dysregulation of essential processes, including extracellular matrix (ECM) remodeling, cell adhesion, and signaling pathways such as PI3K-Akt, ECM-receptor interaction, and focal adhesion. Survival research using the UALCAN platform identified IGF1 as a prospective predictive biomarker. These results underscore the pivotal roles of extracellular matrix dynamics, tumor-stroma interactions, and signaling pathways in facilitating metastatic prostate cancer. The discovered genes and pathways provide intriguing candidates for therapeutic intervention and prognostic evaluation, highlighting the potential of transcriptome-based strategies to enhance precision oncology in metastatic prostate cancer.

Keywords: Prostate Cancer; Bone Metastasis; Differentially Expressed Genes; Protein-Protein Interaction Network; Hub Genes; Extracellular Matrix Remodeling; PI3K-Akt Signaling; Transcriptomics; IGF1; Precision Oncology

INTRODUCTION

Prostate cancer (PCa) is among the most common malignancies in males globally and continues to be a major contributor to cancer-related morbidity and death. Localised prostate cancer is often controlled satisfactorily with surgery, radiation treatment, and androgen deprivation therapy (ADT); nonetheless, a percentage of individuals progress to advanced disease, marked by metastases to distant organs (Baude et al., 2022; Leslie et al., 2025; Rawla, 2019). Bone is the most prevalent and clinically important metastatic location, affecting roughly 80% of men with advanced prostate cancer. Bone metastases result in significant consequences, such as bone pain, pathological fractures, spinal cord compression, and diminished quality of life, eventually leading to heightened death rates (D'Oronzo et al., 2019; Tsuzuki et al., 2016). Notwithstanding improvements in treatment approaches, metastatic prostate cancer mainly remains untreatable, highlighting the need for a more profound comprehension of the biological pathways that propel illness advancement (Abida et al., 2025; Kulasegaran & Oliveira, 2024). The metastatic progression of prostate cancer to the bone is a complex, multi-step process that entails the acquisition of invasive and migratory characteristics by tumour cells, intravasation into circulation, survival in the bloodstream, extravasation into the bone microenvironment, and subsequent colonisation and proliferation (Martin et al., 2013). The bone creates a

unique metastatic environment rich in growth factors, extracellular matrix elements, and stromal cells that engage with prostate cancer cells, facilitating their survival and adaptability. The molecular interaction between tumour cells and bone-resident cells, such as osteoblasts, osteoclasts, and bone marrow stromal cells, promotes the formation of metastatic lesions (Kolb & Bussard, 2019). Factors obtained from tumours drive osteoclast-mediated bone resorption, releasing supplementary growth factors that further facilitate tumour proliferation in a self-sustaining loop referred to as the "vicious cycle" of bone metastases. Nonetheless, the exact molecular pathways regulating this relationship are not well comprehended, hindering the advancement of efficient treatment approaches (Győri & Mócsai, 2020; Shupp et al., 2018). High-throughput gene expression profiling using microarray technology has become a potent instrument for clarifying the molecular framework underlying cancer development. Through the analysis of differentially expressed genes (DEGs) in original prostate cancer tissues versus metastatic bone lesions, researchers may discern essential molecular fingerprints linked to disease progression (Bubendorf, 2001; Liang et al., 2004). Prior research has emphasised the significance of critical oncogenic pathways in the metastasis of prostate cancer, including the androgen receptor (AR) signalling pathway, epithelial-to-mesenchymal transition (EMT), PI3K/Akt/mTOR, Wnt/β-catenin, and transforming growth factor-beta (TGF-β) signalling. These pathways govern critical cellular functions, including tumour growth, invasion, immune evasion, and adaptability to the bone microenvironment. Moreover, variations in gene regulatory networks, including non-coding RNAs and epigenetic changes, may also facilitate metastatic advancement (Baba et al., 2023; de Visser & Joyce, 2023). This work intends to do an extensive gene expression profile and functional analysis of prostate cancer and its metastatic advancement to the bone using microarray data. By identifying differentially expressed genes and conducting pathway enrichment analysis, we aim to elucidate the principal molecular drivers of bone metastasis. Functional enrichment studies, including Gene Ontology (GO) annotation, Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway mapping, and protein-protein interaction (PPI) network analysis, will be used to ascertain the biological relevance of the discovered genes (Karimizadeh et al., 2019). The research will concentrate on discovering possible predictive indicators and treatment targets to enhance clinical outcomes for individuals with metastatic prostate cancer. This study seeks to elucidate the molecular pathways of prostate cancer metastasis to the bone by using high-throughput transcriptomic data and bioinformatics methodologies. This study's results might enhance precision medicine by enabling the creation of tailored medicines that interrupt critical metastasis pathways. A comprehensive knowledge of the molecular changes linked to metastatic prostate cancer may facilitate advancements in diagnostic, prognostic, and therapeutic approaches, therefore improving patient survival and quality of life.

MATERIALS AND METHODS

Data collection

The primary dataset included in this research was obtained from the Gene Expression Omnibus (GEO) database. Stringent keyword restrictions and selection criteria were used to get raw expression profiles of tissue and clinical specimens. The dataset GSE69223 was used to evaluate gene expression data related to prostate cancer, while the dataset GSE14359 was used for bone cancer (osteosarcoma). The GSE69223 dataset comprises tumour and normal samples obtained from 15 patients, with each providing matched samples for a direct comparison of tumour and normal gene expression patterns. Five frozen conventional osteosarcoma samples and two non-neoplastic primary osteoblast cell samples were analysed to identify differentially expressed genes (DEGs) in osteosarcoma. The GEPIA2 online tool was used to assess the differential expression in prostate cancer and osteosarcoma for validation purposes. The overlapping differentially expressed genes (DEGs) discovered in both the GEPIA2 dataset and the GEO datasets were chosen to mitigate the effects of dataset heterogeneity and enhance the reliability of the results. This integrated method enabled a thorough evaluation of the gene expression patterns linked to prostate cancer and its metastatic advancement to bone.

Data Pre-processing

The series matrix files for GSE69223 and GSE14359 were obtained from the GEO dataset to enable a thorough study. Before analysis, probe-level data in each dataset was transformed into standardised gene symbols, matching gene identities with globally accepted nomenclature. To achieve consistency and reduce any technical biases, the datasets were normalised using the robust multiarray average (RMA) method in the R software environment (version 2.6.0).

This normalisation technique standardised gene expression data, maintaining uniformity in size and distribution across the dataset and, hence, improving the trustworthiness of future studies.

Identification of Differentially Expressed Genes (DEGs)

This work identified differentially expressed genes (DEGs) in prostate cancer and osteosarcoma using GEO2R, using a rigorous criteria of p-value < 0.05 and absolute log fold change > 1. The GEO2R analysis produced a volcano plot, which graphically depicts changes in gene expression, with fold change values on the x-axis and statistical significance (p-value) on the y-axis. Gene expression profiles for prostate cancer and osteosarcoma were further acquired from GEPIA2 to enhance this research, using the same criteria for DEG identification. Additionally, FunRich V3.1.3 software was used to analyse DEGS across datasets, facilitating the visualisation of common and distinct molecular targets. The Venn diagrams generated by FunRich elucidated the intersections of genes and pathways, providing a comparative analysis of molecular processes in prostate cancer and osteosarcoma.

Protein-protein interaction and hub gene identification

Protein-protein interaction (PPI) analysis was performed using the STRING database, whereby a list of differentially expressed genes (DEGs) was entered to illustrate a network of both physical and functional relationships. Interactions with a cumulative score over 0.08 were deemed significant, hence affirming the trustworthiness of the identified protein relationships. The PPI network was created and visualised using Cytoscape software (version 3.5.1; http://www.cytoscape.org). The cytoHubba plugin in Cytoscape was used to discover pivotal regulatory genes within the network, with hub genes characterised as nodes exhibiting a degree greater than 10, signifying their centrality in cellular processes. This integrated strategy offers a thorough tool for investigating intricate protein interactions and identifying essential genes implicated in prostate cancer growth and metastasis.

mRNA expression and survival analysis of hub genes

In Silico tools like UALCAN and GEPIA were used to assess mRNA expression levels and their association with patient survival in prostate cancer. A statistically significant level of $p < 0.05$ was used to find the relationship between gene expression levels and patient survival outcomes in a study of survival risk plots. Data pertaining to prostate cancer and osteosarcoma were obtained and used for expression validation. The expression levels were quantified as transcripts per million (TPM) values, which facilitated the categorisation of patients into two groups. Utilising the GEPIA database, patients with TPM levels under the lower quartile were designated as the low/medium expression group, while those with TPM values beyond the upper quartile were classified as the high expression group. The survival analysis and expression connections enabled the identification of key hub genes linked to prostate cancer prognosis, offering significant insights into prospective biomarkers for disease progression and treatment options.

Gene oncology and pathway enrichment analysis

Pathway enrichment studies of differentially expressed genes (DEGs) were performed using Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) via the Database for Annotation, Visualisation, and Integrated Discovery (DAVID) platform (https://david.ncifcrf.gov/tools.jsp). A rigorous significance level of $p < 0.05$ was used to ascertain the discovery of physiologically relevant pathways linked to prostate cancer development. KEGG pathway analysis was used to identify substantially enriched pathways, offering insights into the probable functional functions of DEGs in tumorigenesis and metastatic progression. A pathway crosstalk study was conducted to investigate the interconnections between pathways using established statistical criteria. This standard includes a Benjamini-Hochberg adjusted p-value of <0.05, guaranteeing statistical robustness. A Jaccard coefficient and an overlap coefficient, both established at 50% and surpassing 0.5, were used to ascertain statistically significant route interactions. This thorough examination of DEGs inside certain pathways enhances comprehension of their roles in critical biological processes and regulatory networks. This work elucidates essential molecular pathways and their interrelations, offering vital insights into the processes of prostate cancer development and its metastatic advancement to the bone.

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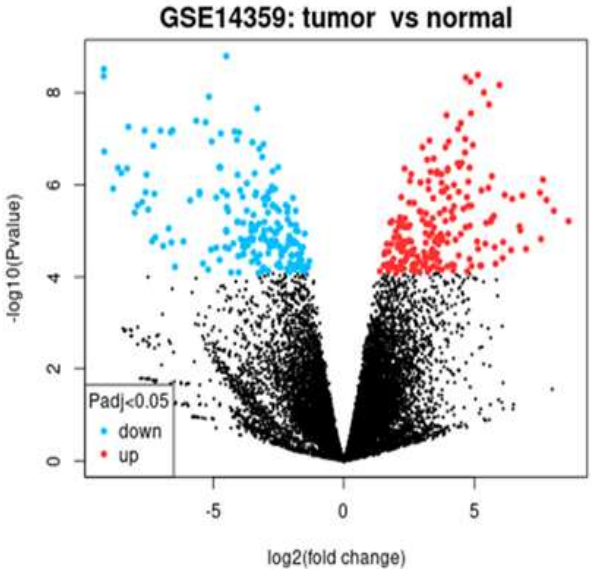
RESULT

Identification of DEGs in Prostate cancer and Osteosarcoma

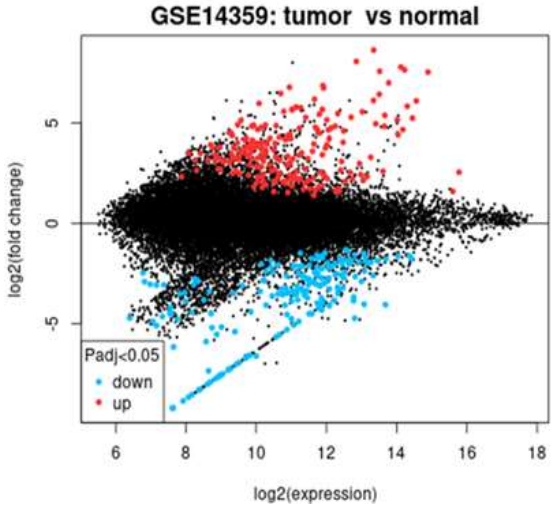
The identification of differentially expressed genes (DEGs) in prostate cancer and osteosarcoma was performed using GEO2R analysis, using the limma software to compare gene expression levels between malignant and normal tissue samples. Rigorous selection criteria were used, including an adjusted p-value < 0.05 and a log fold change > 1, so guaranteeing that only statistically significant and physiologically relevant differentially expressed genes (DEGs) were included. This approach enabled the creation of volcano maps to illustrate the distribution of differentially expressed genes across datasets. Analysis of the GSE69223 dataset for prostate cancer revealed a total of 1326 differentially expressed genes (DEGs) (Figures 3A and 3B). These genes demonstrated substantial differential expression between tumour and normal samples. The volcano graphic illustrates the most significantly upregulated and downregulated genes, facilitating the identification of crucial molecular contributors to prostate cancer growth. Figure 2 depicts the most regulated genes in prostate cancer.

Similarly, the analysis of the dataset GSE14359 for osteosarcoma identified 375 differentially expressed genes (DEGs) using the same stringent statistical criteria (Figure1A and1B). These DEGs elucidate the molecular pathways involved in osteosarcoma aetiology and provide possible treatment targets. The thorough identification of DEGs in prostate cancer and osteosarcoma provides a comparative framework to investigate shared and distinct molecular changes linked to both cancers. This work provides a basis for further research on common oncogenic pathways, prospective biomarkers, and focused therapy approaches for both cancer types.

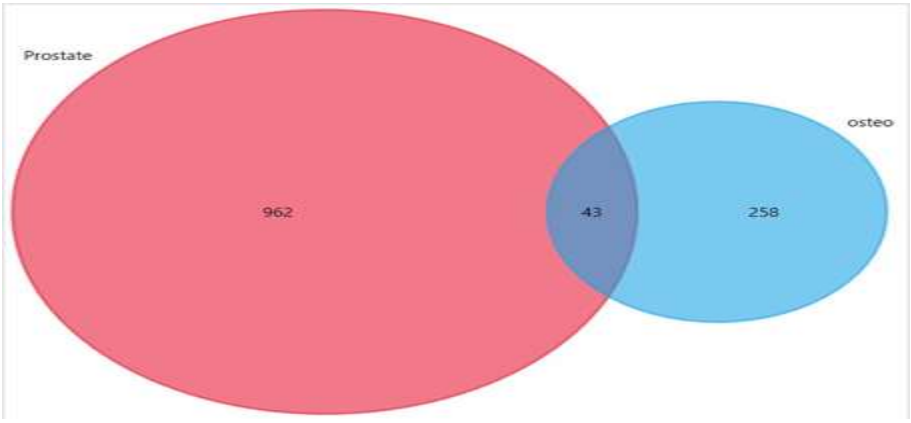
1A



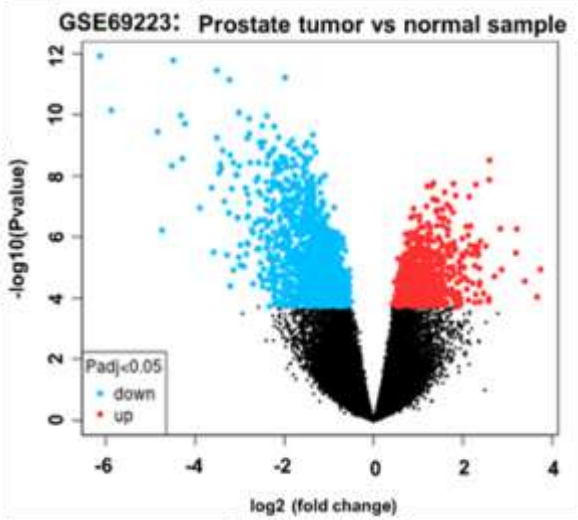
1B

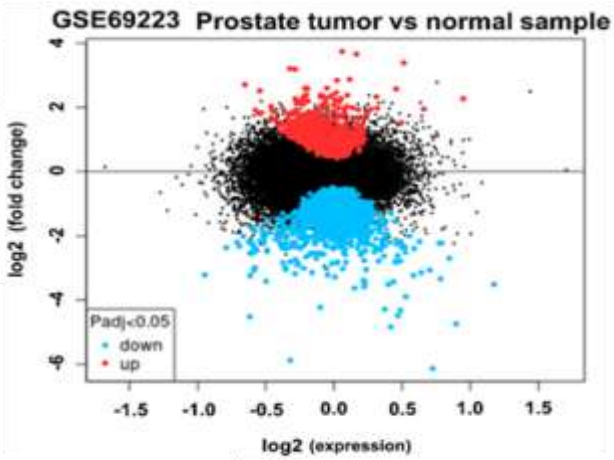


2.

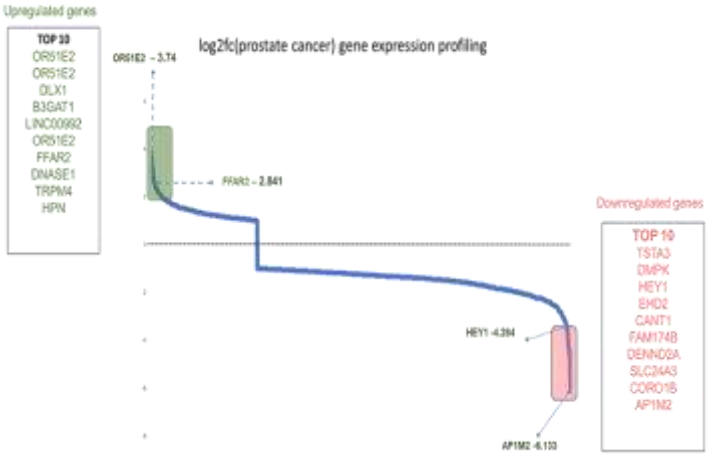

Prostate
osteo
962
43
258

3A **3B**


GSE69223: Prostate tumor vs normal sample
-log10(Pvalue)
log2 (fold change)
Padj<0.05
down
up


GSE69223 Prostate tumor vs normal sample
log2 (fold change)
log2 (expression)
Padj<0.05
down
up

4

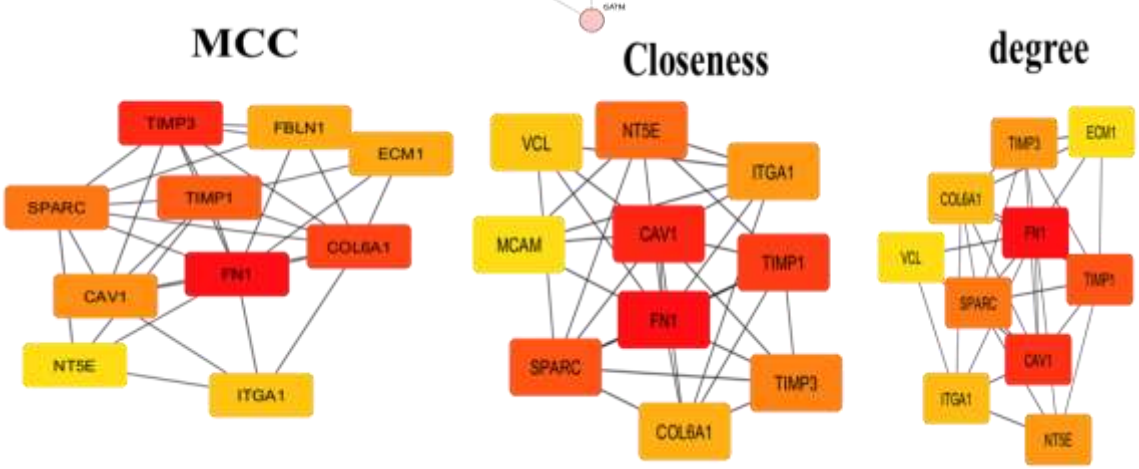


Protein-Protein Interaction (PPI) Network Construction and Hub Gene Identification
The protein-protein interaction (PPI) network was constructed using STRING analysis based on proteins encoded by differentially expressed genes (DEGs). In the first analysis, 43 overlapping DEGs were evaluated, and it was observed that 42 of them were interconnected within the network. Similarly, (Figure 5A) a broader analysis identified 70 out of 1326 DEGs forming significant interactions within the PPI network, indicating potential molecular associations in prostate cancer progression. The network was further visualized using Cytoscape software for a clearer representation of interactions. To identify key regulatory genes within the network, hub gene analysis was performed using methods such as Maximum Clique Centrality (MCC), degree, and closeness centrality (Figure 5B). As a result, multiple hub genes were identified. The first set of hub genes included TIMP3, CAV1, COL6A1, ECM1, FBLN1, NT5E, MCAM, ITPR1, and VCL, which may play crucial roles in the metastatic progression of prostate cancer.

Additionally, another set of key hub genes, IGF1, FGF2, and EGF, was identified (Figure 6A). Notably, EGF was upregulated among the DEGs, while IGF1 and FGF2 were downregulated, suggesting their significant involvement in tumor progression and metastatic adaptatio (Figure 6B and 6C). The integration of these findings provides a comprehensive understanding of the molecular interactions within prostate cancer and its metastatic progression. The identified hub genes serve as potential biomarkers and therapeutic targets, warranting further investigation to elucidate their functional roles in disease development.

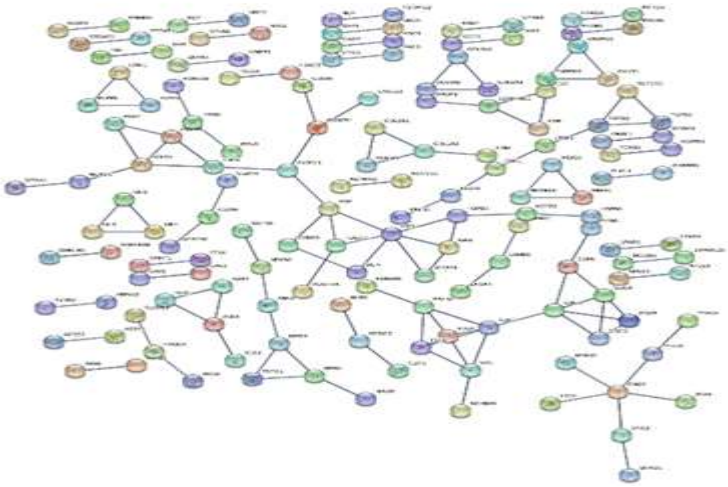
5A

5B


MCC
TIMP3
FBLN1
ECM1
SPARC
TIMP1
COL6A1
CAV1
FN1
NT5E
ITGA1
Closeness
VCL
NT5E
ITGA1
MCAM
CAV1
TIMP1
FN1
SPARC
TIMP3
COL6A1
degree
TIMP3
ECM1
COL6A1
FN1
VCL
TIMP1
SPARC
CAV1
ITGA1
NT5E

6A

6B



6C

B

MCC	Degree	Closeness
COL6A2	GNG2	IGF1
COL6A3	COL1A2	FGF2
COL1A2	EGF	EGF
COL6A1	IGF1	CAV1

COL4A1	COL6A1	DCN
COL5A2	FGF2	COL1A2
COL5A1	DCN	CXCL12
FBN1	ITGA1	FGF7
FGF2	FBN1	PDGFRA
EGF	VCL	COL6A1

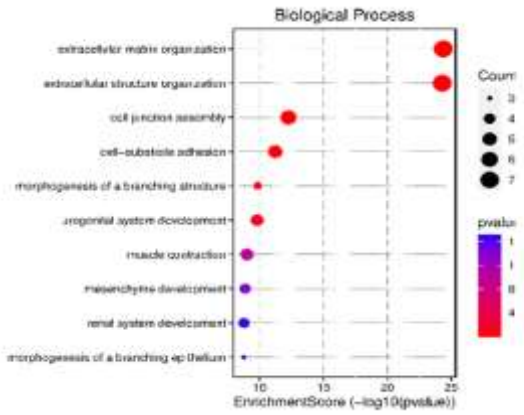
Gene ontology and KEGG pathway analysis of DEGs

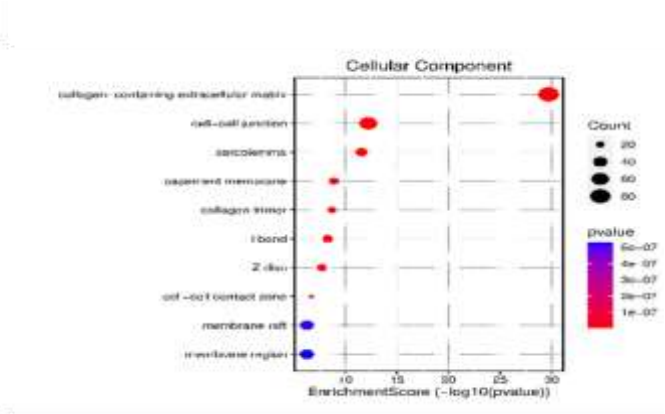
The comprehensive analysis of differentially expressed genes (DEGs) through Gene Ontology (GO) and the Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways provides profound insights into the intricate molecular mechanisms underlying the progression of prostate cancer, particularly its metastatic spread to bone tissue. The GO analysis categorises DEGS into three primary domains: biological processes (BP), cellular components (CC), and molecular functions (MF), each of which sheds light on key aspects of tumour progression and metastatic potential. The biological process enrichment analysis (BP) highlights the significance of the extracellular matrix's (ECM) organisation and structural assembly, emphasising their crucial role in tumour invasion and metastasis. Enrichment of biological processes such as cell adhesion, cell junction assembly, and morphogenesis underscores the dynamic interactions between tumour cells and the bone microenvironment, which are essential for metastatic dissemination. The ECM plays a vital role in remodelling the tumour's microenvironment by facilitating cancer cell migration, invasion, and establishment in distant metastatic sites. At the cellular component (CC) level, the analysis identifies key structural components involved in cancer progression, including the collagen-containing extracellular matrix, cell-cell junctions, and basement membranes. These findings suggest significant alterations in tissue architecture and cellular interactions, characteristics of aggressive metastatic cancer . The remodelling of the ECM and the disruption of normal tissue integrity are key hallmarks of bone metastasis, which allows tumour cells to establish a supportive microenvironment for sustained growth and survival. The molecular function (MF) analysis provides further insights into the mechanisms by which tumour cells interact with and modify their surrounding environment. Functions such as extracellular matrix structural constituent activity, glycosaminoglycan and collagen binding, and heparin and actin binding are significantly enriched, highlighting the crucial role of ECM components in modulating tumour-stroma interactions, cell adhesion, and migration. These molecular functions are essential for cancer cells to navigate through the extracellular environment, evade immune responses, and establish secondary tumour sites in the bone. Pathway enrichment analysis using the KEGG database reveals dysregulation in critical signalling pathways that contribute to tumour progression and metastasis. Among the most significantly enriched pathways are focal adhesion, ECM-receptor interaction, and proteoglycans in cancer, all of which play essential roles in tumour cell survival, migration, and invasion. Additionally, the dysregulation of key oncogenic signalling pathways, including PI3K-Akt and Rap1 signals, further supports their involvement in promoting metastatic potential. These pathways regulate crucial cellular processes such as proliferation, survival, angiogenesis, and immune evasion, all of which are fundamental to tumour progression. Interestingly, pathway analysis also reveals potential links between prostate cancer metastasis and cardiomyopathy-related pathways, suggesting shared molecular mechanisms between cancer progression and cardiovascular dysfunction. These findings highlight the need for a comprehensive approach to patient care, considering the potential systemic effects of prostate cancer metastasis and its treatment. In summary, the integration of GO and KEGG pathway analyses provides a detailed molecular perspective on the biological processes, cellular components, and molecular functions associated with bone metastasis in prostate cancer. The findings underscore the importance of tumour-microenvironment interactions in facilitating metastatic spread and highlight potential therapeutic targets. Targeting dysregulated pathways, such as focal adhesion, ECM receptor interaction, and PI3K/Akt signalling, presents promising avenues for the development of novel treatment strategies aimed at halting or slowing

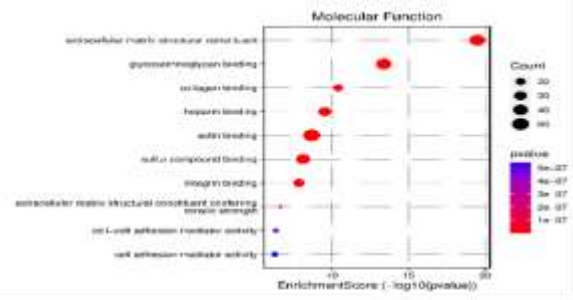
disease progression. Understanding these molecular underpinnings offers significant potential for improving patient outcomes and developing targeted interventions tailored to metastatic prostate cancer.

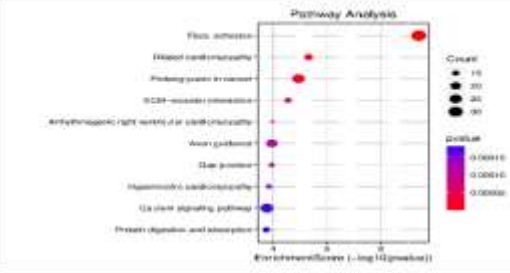
7A and 7B

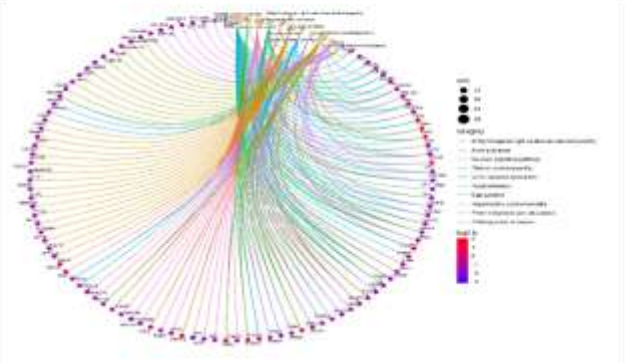
7C and 8

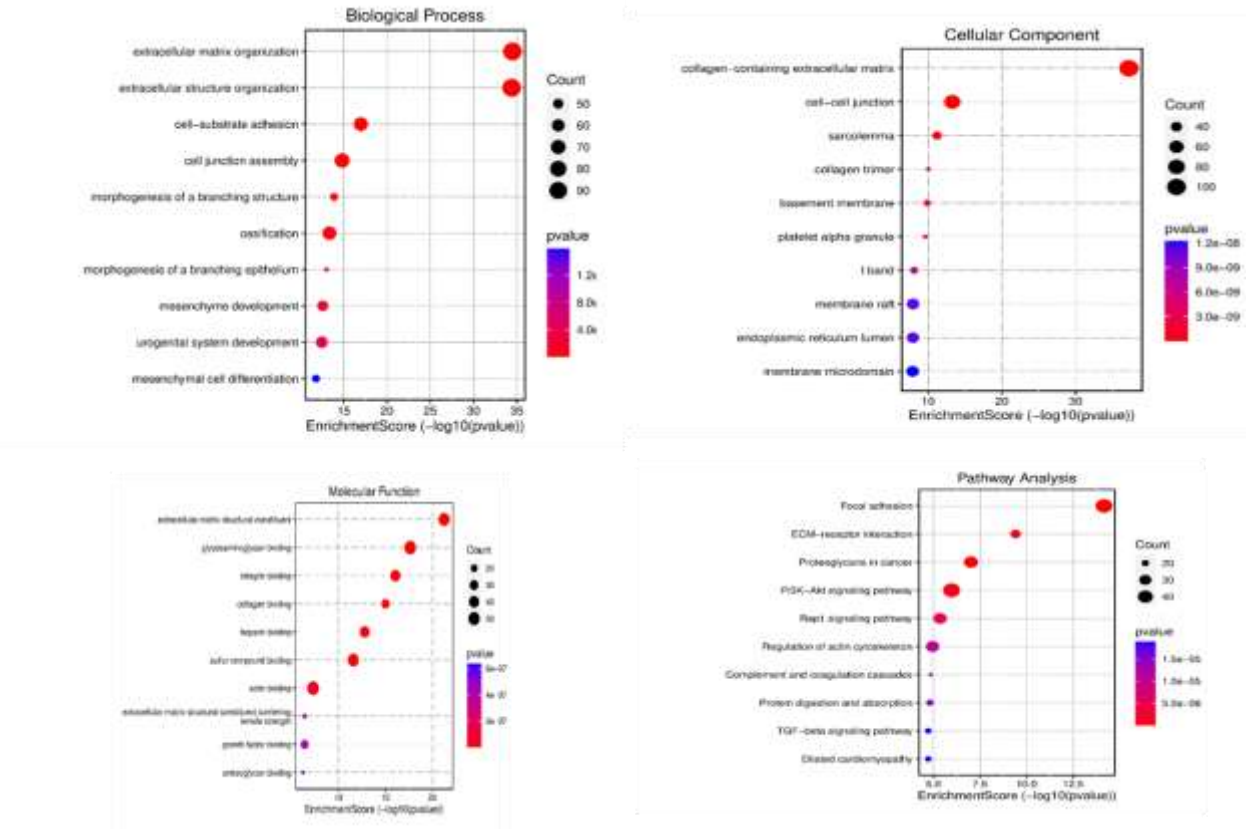


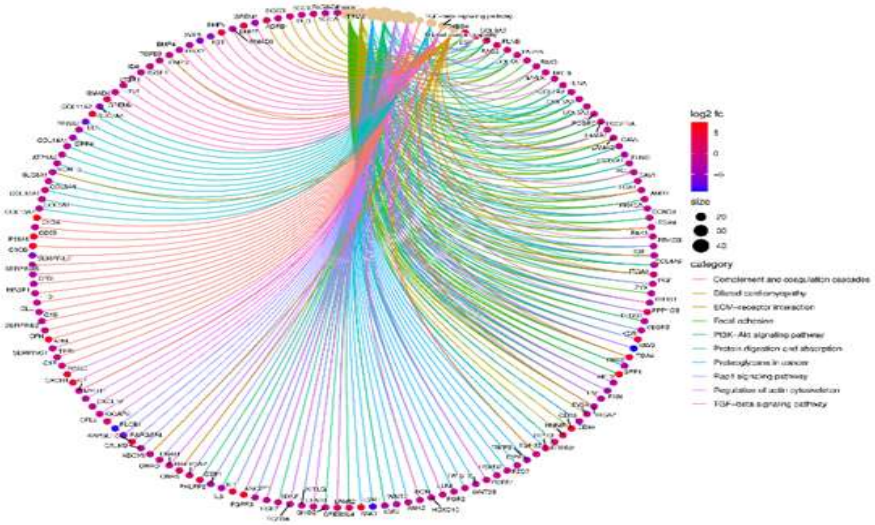






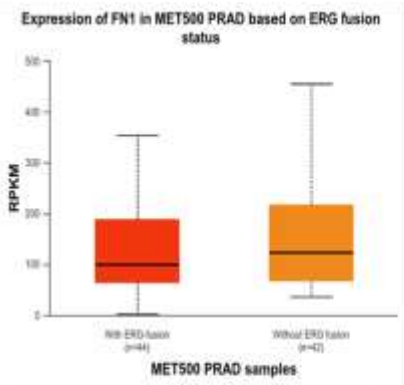


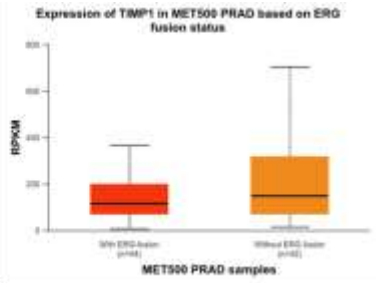

Biological Process
extracellular matrix organization
extracellular structure organization
cell-substrate adhesion
cell junction assembly
morphogenesis of a branching structure
ossification
morphogenesis of a branching epithelium
mesenchyme development
urogenital system development
mesenchymal cell differentiation
Count
pvalue
EnrichmentScore (−log10(pvalue))
Cellular Component
collagen-containing extracellular matrix
cell–cell junction
sarcolemma
collagen trimer
basement membrane
platelet alpha granule
I band
membrane raft
endoplasmic reticulum lumen
membrane microdomain
Count
pvalue
EnrichmentScore (−log10(pvalue))
Molecular Function
Pathway Analysis
Focal adhesion
ECM-receptor interaction
Proteoglycans in cancer
PI3K-Akt signaling pathway
Rap1 signaling pathway
Regulation of actin cytoskeleton
Complement and coagulation cascades
Protein digestion and absorption
TGF-beta signaling pathway
Dilated cardiomyopathy
Count
pvalue
EnrichmentScore (−log10(pvalue))

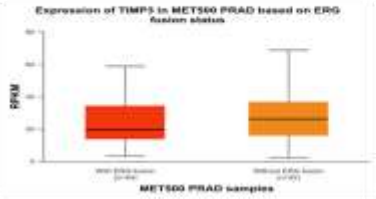

log2 fc
size
category
Complement and coagulation cascades
Dilated cardiomyopathy
ECM-receptor interaction
Focal adhesion
PI3K-Akt signaling pathway
Protein digestion and absorption
Proteoglycans in cancer
Rap1 signaling pathway
Regulation of actin cytoskeleton
TGF-beta signaling pathway

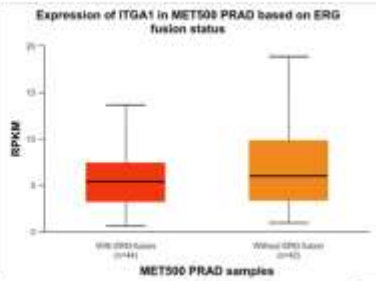
Gene symbol	Gene title	log2(fold change)	log10 p value	Chromosome number
HBB	hemoglobin subunit beta	8.618	5.21	chr11:5,225,464-5,229,395
HLA-DRA	major histocompatibility complex, class II, DR alpha	8.052	5.434	chr6:32,439,878-32,445,046
HBA1	hemoglobin subunit alpha 1	7.77	5.671	chr16:176,680-177,522
HBA2	hemoglobin subunit alpha 2	7.635	6.111	chr16:172,876-173,710
HBA2	hemoglobin subunit alpha 2	7.559	4.82	chr16:172,876-173,710
SPP1	secreted phosphoprotein 1	7.521	5.827	chr4:87,975,667-87,983,532
HBA1	hemoglobin subunit alpha 2	6.985	4.604	chr16:176,680-177,522
C1QA	complement C1q A chain	6.85	5.767	chr1:22,635,077-22,639,678
RGS1	regulator of G-protein signaling 1	6.767	5.009	chr1:192,575,763-192,580,024
MMP13	matrix metallopeptidase 13	6.732	5.096	chr11:102,942,995-102,955,732
MS4A4A	membrane spanning 4-domains A4A	6.457	5.695	chr11:60,185,657-60,318,080
HLA-DRA	major histocompatibility complex, class II, DR alpha	6.413	4.581	chr6:32,439,878-32,445,046
RGS2	regulator of G-protein signaling 2	6.17	5.782	chr1:192,809,039-192,812,275
RNASE1	ribonuclease A family member 1, pancreatic	6.108	4.756	chr14:20,801,228-20,802,855
HBA2	hemoglobin subunit alpha 2	6.09	4.405	chr16:172,876-173,710
SNX10	sorting nexin 10	5.965	8.171	chr7:26,291,862-26,374,383
HBA2	hemoglobin subunit alpha 2	5.821	4.287	chr16:172,876-173,710

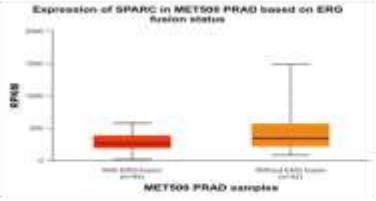
FGFR3	fibroblast growth factor receptor 3	5.785	4.641	chr4:1,793,293-1,808,872	
MRC1	mannose receptor, C type 1	5.749	5.321	chr10:17,809,348-17,911,164	
GPM6B	glycoprotein M6B	5.677	6.187	chrX:13,770,939-13,938,638	

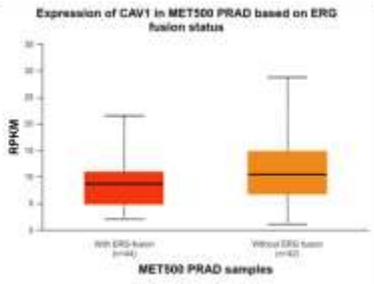


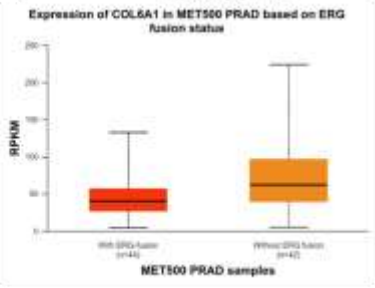












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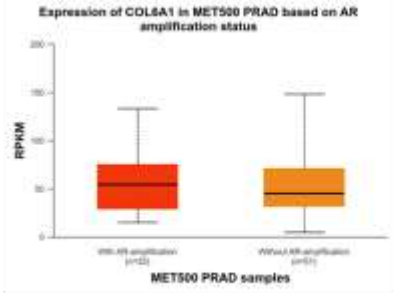

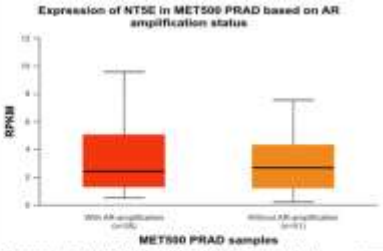

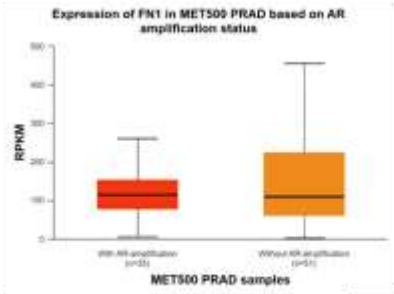

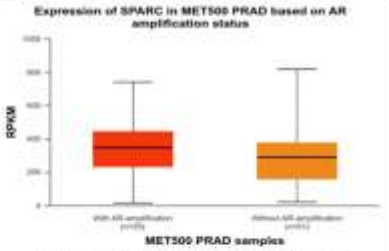

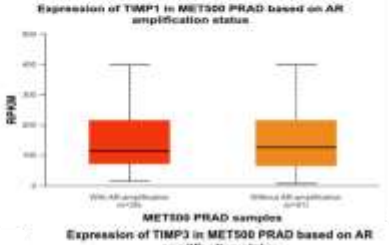

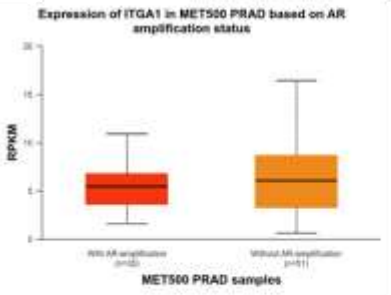

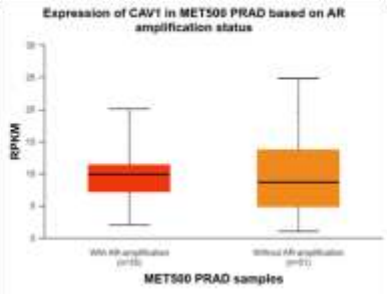

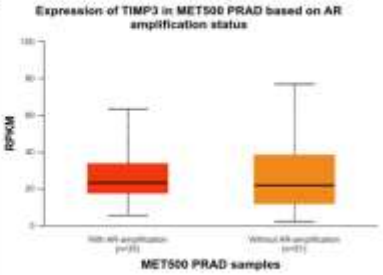

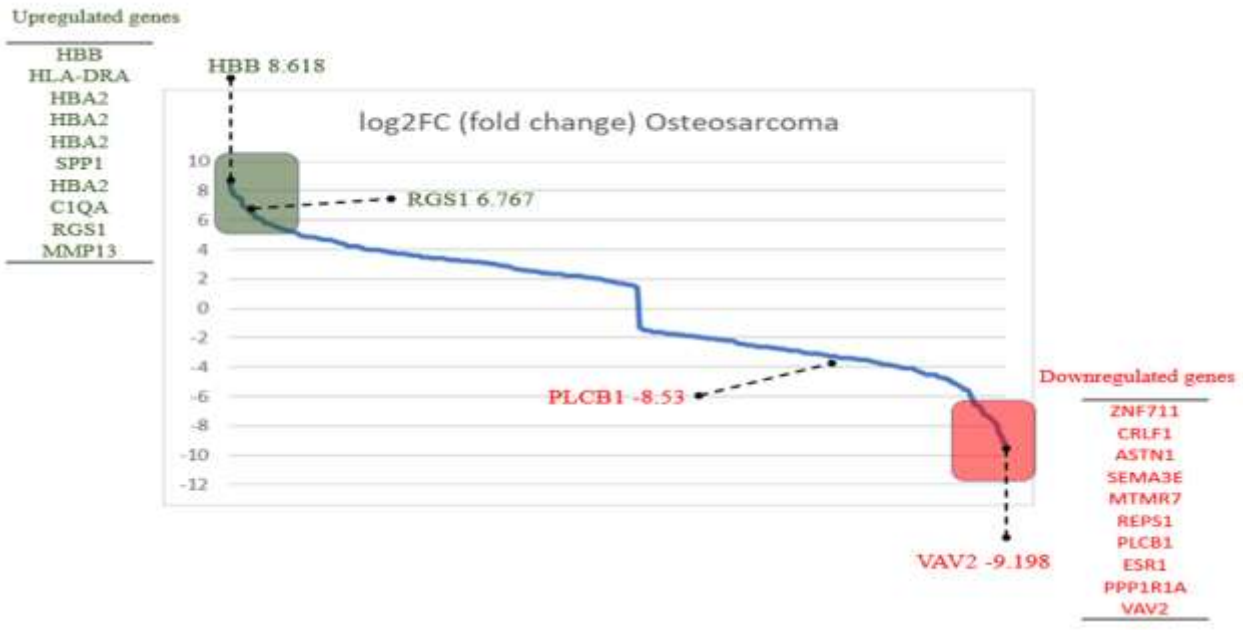

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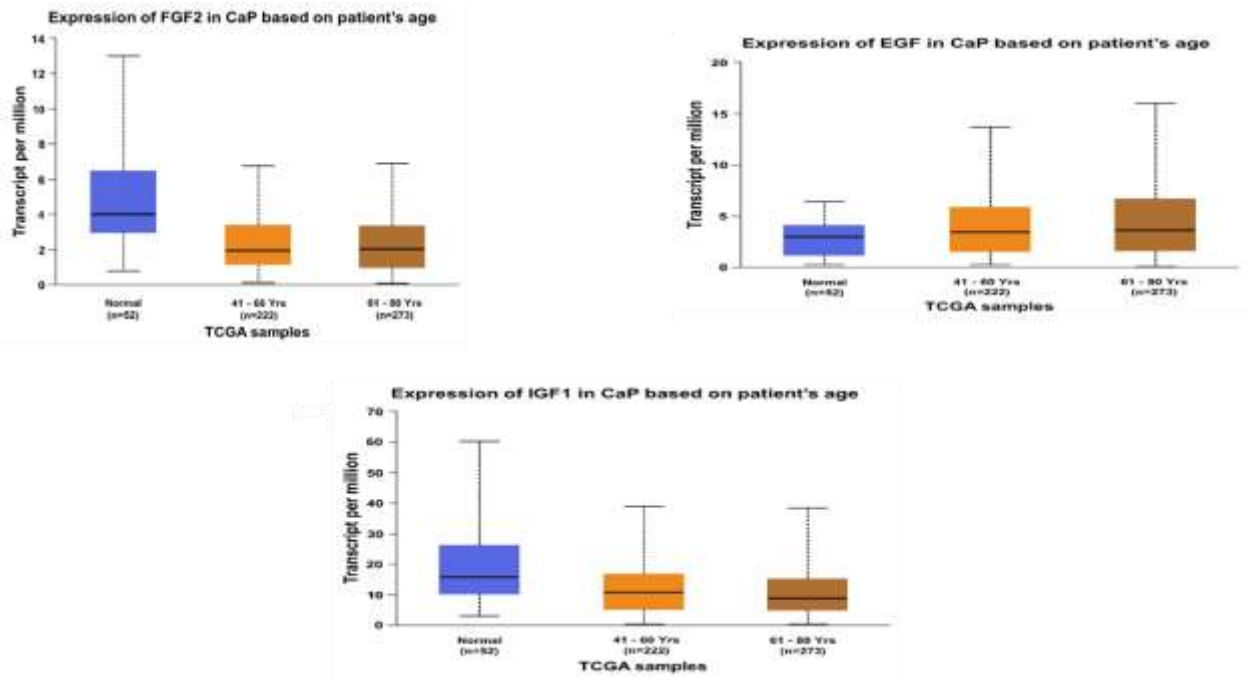
Verification and Survival Analysis of Hub Genes in Prostate Cancer

To thoroughly understand the molecular dynamics of prostate cancer, it is essential to investigate the validation and survival analysis of hub genes, highlighting key aspects that affect disease development. This work used the UALCAN web server, known for its expertise in cancer data processing, to examine three critical hub genes (Figures 9A and 9B). Certain genes have considerable downregulation in prostate cancer patients relative to their normal counterparts, as seen in (Figure10). FGF2 and IGF1 exhibit downregulation, whereas EGF shows overexpression, indicating their possible involvement in cancer development. Survival study, based on the importance of p-values, demonstrates varying prognostic effects across these hub genes. IGF1 shows marginal significance ($p = 0.097$), suggesting a potential role in prostate cancer prognosis, although FGF2 and EGF provide non-significant p-values ($p = 0.8$ and $p = 0.89$, respectively), showing little predictive value regarding disease outcome, as seen in figure 9. Although FGF2 and EGF lack statistical significance, a further examination of their biological activities may provide insights into their roles in prostate cancer development. Such research provides potential for clarifying new pathways for therapeutic intervention and prognostic evaluation in the treatment of prostate cancer.

9A



9B

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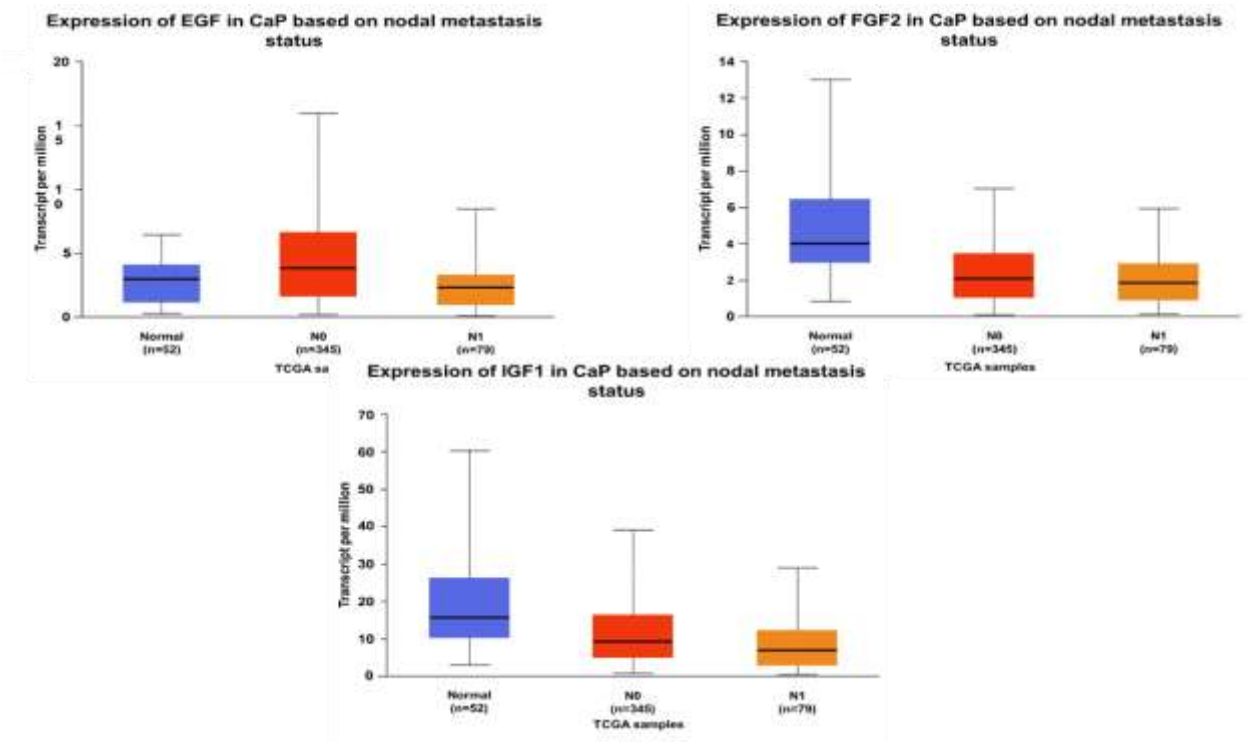

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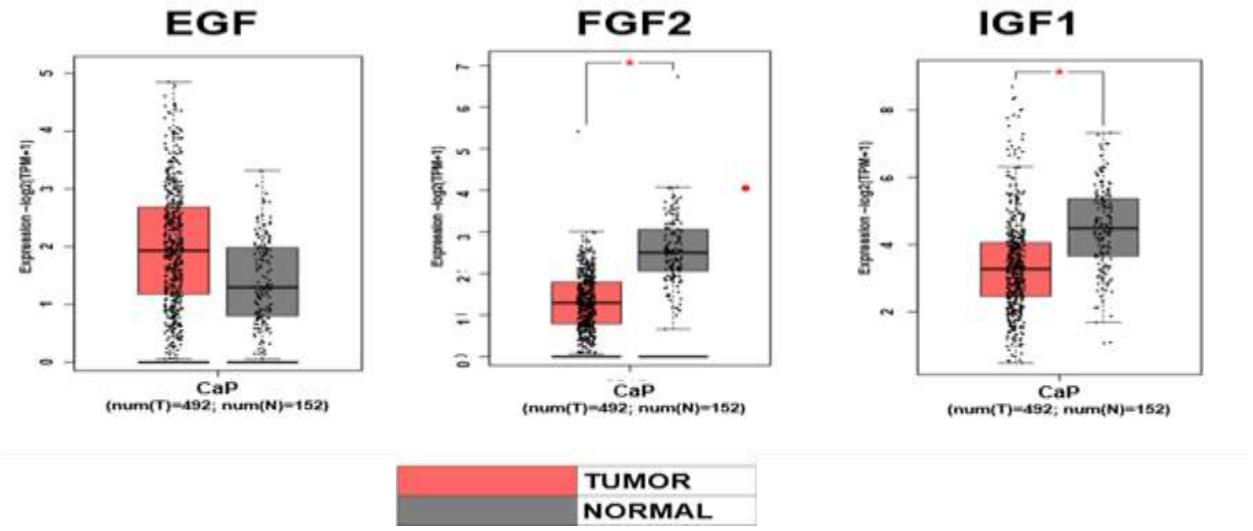

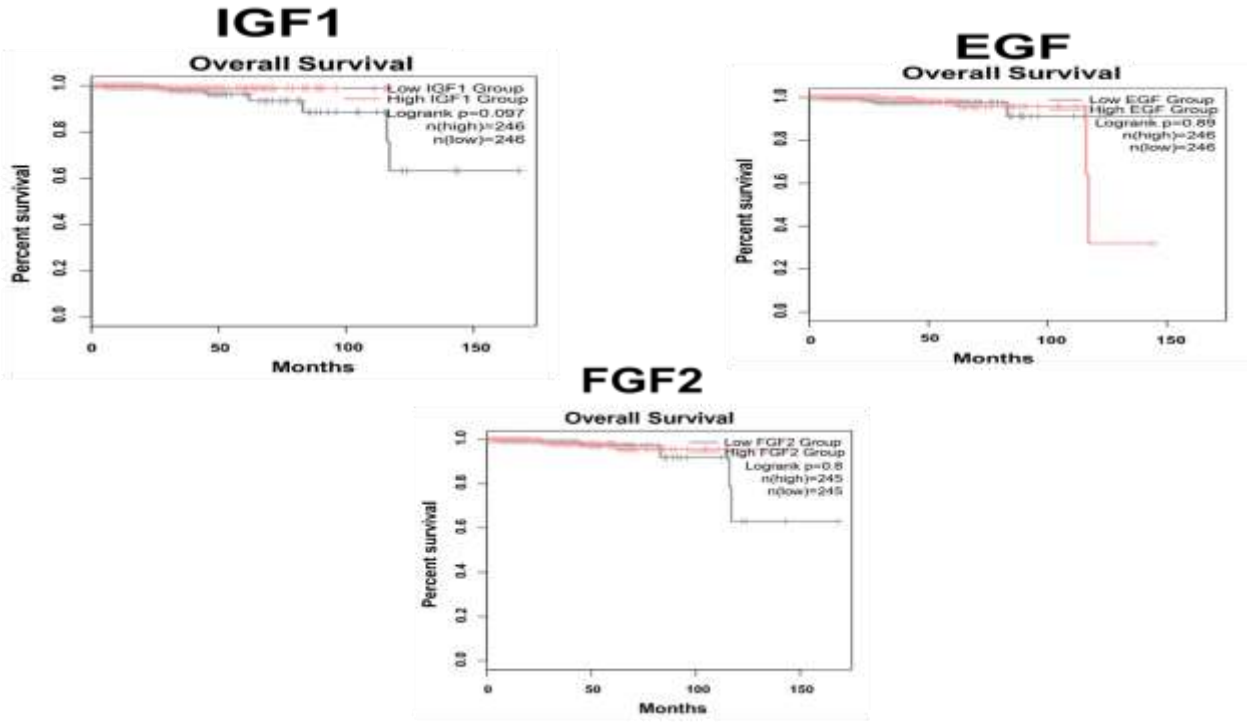

DISCUSSION

This work offers substantial insights into the molecular pathways that drive prostate cancer (PCa) growth and its metastatic transfer to bone. Utilising high-throughput microarray data and bioinformatics studies, we identified critical differentially expressed genes (DEGs), significant signalling pathways, and vital protein–protein interactions related to this metastatic process. These findings are in line with earlier research that showed how important oncogenic pathways, extracellular matrix remodelling, and tumour-stroma interactions are for encouraging bone metastases. The Gene Ontology (GO) enrichment study yielded more insights into the biological processes implicated in prostate cancer metastasis. The enhancement of extracellular matrix organisation, cellular adhesion, and morphogenesis highlights the significance of ECM remodelling in promoting cancer cell invasion and spread (Parihar et al., 2022; Samaržija & Konjevoda, 2023). The cellular component analysis highlighted changes in the basement membrane and intercellular connections, indicating substantial structural variations in tumour tissues relative to healthy tissues (Parihar et al., 2022; Rozario & DeSimone, 2010; Saraji et al., 2022; Stewart et al., 2004). The molecular function study emphasised the role of ECM structural components, glycosaminoglycan binding, and actin binding, all of which are essential for sustaining the metastatic capabilities of prostate cancer cells. KEGG pathway enrichment analysis confirmed these results, highlighting the participation of essential signalling pathways, including focal adhesion, ECM-receptor interaction, and PI3K/Akt signalling (Walker et al., 2018; Xie et al., 2022; Yazdani et al., 2024). The activation of these pathways is recognised to facilitate tumour growth by augmenting survival, migratory, and invasion capacities. Our results corroborate earlier research, indicating that constituents, including collagen fibronectin, engage with integrins and growth factor receptors to promote tumour proliferation and metastasis (Hamidi & Ivaska, 2018). Furthermore, our detection of dysregulated proteoglycans in cancer underscores their potential function in influencing the tumour microenvironment and facilitating metastatic colonisation in bone. Previous studies have thoroughly elucidated the molecular mechanisms behind prostate cametastasisingtasising to the bone, emphasising the "vicious

cycle" theory. This idea posits that prostate cancer cells release osteolytic molecules, including parathyroid hormone-related protein (PTHrP) and interleukins, which promote osteoclast-mediated bone resorption (W. Wang et al., 2019). The subsequent liberation of growth factors from the bone matrix, including transforming growth factor-beta (TGF-β) and insulin-like growth factors (IGFs), further amplifies tumour proliferation, establishing a self-sustaining cycle. Our results substantiate this notion by pinpointing critical genes and pathways linked to ECM remodelltumourtumour-microenvironment interactions, and osteocactivation (Linkhart et al., 1996; Sato et al., 2008).

Moreover, our findings underscore the significance of epithelial-to-mesenchymal transition (EMT) in the metastasis of prostate cancer. Prior research has shown that epithelial-mesenchymal transition (EMT), marked by the reduction of epithelial markers such as E-cadherin and the acquisition of mesenchymal markers like N-cadherin and vimentin, promotes the detachment and invasion of cancer cells. Our finding of notable ECM-related alterations indicates that EMT-associated modifications are pivotal in the metastasis process (Liaghat et al., 2024; Montanari et al., 2017). This study's discovery of crucial regulatory genes and signalling pathways has substantial significance for the development of targeted therapeutics for metastatic prostate cancer. The PI3K/Akt pathway has been thoroughly investigated as a therapeutic target, with several inhibitors now undergoing clinical studies. Interventions aimed at ECM-receptor interactions and focal adhesion kinase (FAK) signalling may provide innovative approaches to impede metastatic development(Hashemi et al., 2023; R. Wang et al., 2024). The dysregulation of IGF1 and FGF2 indicates that addressing growth factor signalling may be an effective strategy for reducing prostate cancer metastases to bone. The results of this investigation may possess prognostic significance. A survival study of hub genes indicated that IGF1 expression was somewhat correlated with patient outcomes, indicating its potential as a predictive biomarker. Despite FGF2 and EGF lacking substantial prognostic significance in this research, their role in the metastatic path needs additional confirmation in larger patient populations (Kimura et al., 2010; Liu et al., 2023). Although our work offers significant insights into the genetic landscape of metastatic prostate cancer, certain limitations must be addressed. The dependence on publicly accessible microarray datasets poses possible biases associated with sample heterogeneity and technological variability. Incorporating RNA sequencing (RNA-seq) data and doing in vitro and in vivo functional validations might enhance the results. The work mostly emphasises bioinformatics analysis; hence, experimental confirmation via gene knockdown or overexpression experiments is essential to verify the functional functions of discovered DEGs in prostate cancer metastasis. Future investigations should examine the function of non-coding RNAs, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), in modulating the metastatic process. Furthermore, single-cell transcriptomic methodologies may provide a more nuanced comprehension of tumour heterogeneity and the dynamic interactions between prostate cancer cells and the bone microenvironment.

CONCLUSION

This work thoroughly clarifies the alterations in gene expression and molecular pathways linked to the evolution of prostate cancer and its metastatic adaptation to bone tissue. The discovery of pivotal hub genes, carcinogenic pathways, and extracellular matrix remodelling processes offers significant insights into prospective treatment targets and prognostic indicators. Integrating bioinformatics methodologies with experimental validation may facilitate advancements in diagnostic and therapeutic tactics, hence improving patient outcomes in metastatic prostate cancer.

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