Prominin-1 (PROM1) Significantly Correlated With Bone Metastasis and Influenced Prognosis in Breast Cancer

Yi-nan Wu

Zhejiang University School of Medicine

Cheng-Cheng Yu

Zhejiang University School of Medicine

Kai-min Hu

Zhejiang University School of Medicine

Su-zhan Zhang (✉ zhangsuzhanzd@163.com)

Zhejiang University School of Medicine <https://orcid.org/0000-0002-3199-6547>

Research

Keywords: breast cancer, bone metastasis, DEGs, survival, PROM1

Posted Date: October 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-951336/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

This study aimed to explore the important biomarker associated with bone metastasis (BM) in breast cancer (BRCA).

Methods

The GSE175692 dataset was used to detect significant differential expressed genes (DEGs) between BRCA samples with or without BM, and important pathways were then explored. Further, we constructed protein-protein interaction (PPI) network on GEGs and filtered 5 vital nodes. Through performing cox regression, Kaplan-Meier analysis, nomogram, ROC curve, and risk score model, significant prognostic factor was gradually identified. Finally, gene set enrichment analysis (GSEA) analysis was performed to reveal the potential mechanism.

Results

Totally, 74 DEGs were detected, which mainly correlated with infectious disease, signaling molecules and interaction. The 5 important DEGs were filtered, and cox regression further showed that prominin-1 (PROM1) and C-C Motif Chemokine Ligand 2 (CCL2) were prognosis-related factors. A negative correlation was observed between the expression of these 2 genes and the overall survival of metastatic BRCA patients. Especially, PROM1 presented a better prognostic performance on the survival probability of patients. Prognosis verification analysis also confirmed the significant influence of PROM1 on patient's survival. In addition, we found that PROM1 expression was related to the distant metastasis-free survival in BRCA. Finally, GSEA analysis revealed that PROM1 was negatively related to IGF1 and mTOR pathways involved in BRCA metastasis.

Conclusion

PROM1 was identified as an important DEGs associated with BRCA bone metastasis. It acted as a vital prognostic biomarker involved in BRCA metastasis, which may be due to the negative regulation of IGF1 and mTOR pathways.

1. Introduction

Metastasis accounted for more than 90% of cancer-related mortality [1]. Bone is one of the most preferred sites of metastatic spread from different cancer types, including breast cancer (BRCA). BRCA has become a critical health care issue that substantially affected women worldwide. Besides lung, liver and brain, the most common site for metastasis in BRCA is the bone [2]. Bone is also the first site of distant metastasis in 25% to 40% of patients with advanced BRCA [3]. It should be noted that different subtypes of BRCA exhibited distinct metastatic behavior in terms of kinetics and anatomic sites of relapse [4, 5]. For example, bone-only metastases were more common in the HR-positive group than in the other subtypes

[6]. The bone metastasis in BRCA was able to influence the survival of patients. It was reported that 5-year survival rate of BRCA patients without metastasis was greater than 95% but close to only 20% once bone metastasis occurred [7]. In addition, BRCA bone metastasis also caused series of bone-related complications such as pain, pathologic fractures and spinal cord compression, which significantly affected the patient's quality of life [6], also increased the medical costs and mortality risk. As such, new targets and therapeutic strategies associated with BRCA bone metastasis are urgently required.

Over the past few decades, a great deal of biomarkers about BRCA bone metastasis has been generated, which facilitated the studies on cancer etiology. Pantano et al. found that integrin alpha5 (ITGA5) was highly expressed in bone metastases, compared to lung, liver, or brain metastases [8]. Awolaran et al. identified 15 proteins expressed by BRCA cells as factors that mediated BRCA bone metastasis, and upregulation of them could promote BRCA metastasis to bone, except for C-C Motif Chemokine Ligand 2 (CCL2) showed a reduced expression [7]. Westbrook et al. identified and validated the dedicator of cytokinesis protein 4 (DOCK4) as a potential biomarker for risk of bone metastasis development in patients with early BRCA [9]. In addition, Zhang et al. found that microRNA-429 can inhibit BRCA bone metastasis by regulating matrix metalloproteinase 9 (MMP-9) [10]. More and more useful biomarkers should be detected to reveal the cancer etiology involved in BRCA bone metastasis.

The present study was conducted to detect significant bone metastasis-associated biomarkers involved in BRCA. The differential expressed genes (DEGs) between BRCA bone metastasis and non-metastasis samples were initially screened out through the GEO dataset. Then, the impacts of significant DEGs on the survival probability of metastasis BRCA samples were predicted. Regarding significant prognostic biomarkers, we explored associated regulatory pathways involved in BRCA metastasis. This study was conducive to reveal useful biomarkers and potential mechanisms involved in BRCA bone metastasis.

2. Methods

2.1 DEGs identification and function analysis

GSE175692 dataset was used to detect significant differential expressed genes (DEGs), which contained 33 BRCA bone metastasis samples and 151 non-bone metastasis samples. According to the threshold of $P < 0.05$ and absolute log fold change (FC) > 1 , the DEGs between the 2 groups were identified by the limma R package. And top 20 up and down-regulated DEGs were presented. Subsequently, the significant KEGG pathway and GO terms about DEGs were explored through clusterProfiler R package. GO terms were annotated through the biological process (BP), cellular component (CC) and molecular function (MF) aspects.

2.2 PPI network construction and hub genes determination

The protein-protein interaction (PPI) network among DEGs was constructed using the online tool String with setting interacting score as medium, followed by visualization through Cytoscape. The top 10 hub genes among the whole PPI network were detected by the Degree gene ranking method using Cytohubba

plug-in of Cytoscape. Venn analysis was then performed to filter more important nodes between the top 10 hub genes and top20 DEGs. Finally, 5 significant nodes were identified for further investigation.

2.3 Expression analysis on hub genes

The mRNA differential expression of 5 hub genes between BRCA samples with or without bone metastasis was firstly evaluated using the dataset of GSE175692. Then, the GSE14020 dataset was used to verify their mRNA expression, which contained 18 bone metastasis and 47 non-bone metastasis BRCA samples. We also investigated their mRNA expression between normal and BRCA samples based on TCGA data in the GSCA database. In addition, protein expression of hub genes in BRCA was also evaluated based on CPTAC data in UALCAN.

2.4 Prognosis analysis on hub genes

The impact of 5 hub genes on the survival probability of BRCA metastasis samples was initially evaluated using the GSE175692 dataset through univariate cox regression analysis. Kaplan-Meier analysis was performed to explore the influence of hub genes on the overall survival time of BRCA metastasis samples. Further, nomogram and receiver operating characteristic (ROC) curve analyses were conducted to reveal the prognostic performance of hub genes. A risk score model contained hub genes were also constructed to assess the potential prediction ability. Moreover, the prognostic impact of hub genes on the overall survival of BRCA patients was also verified using the GSE124647 dataset, which included 140 BRCA metastasis samples. Regarding the significant prognosis factor, its prognostic impact on distant metastasis-free survival of BRCA patients was verified as well using the GSE11121 dataset, which contained 200 BRCA samples.

2.5 GSEA analysis on significant prognostic factors

Due to the differential expression and vital prognostic effect of significant prognostic factors in metastasis BRCA samples, we performed the gene set enrichment analysis (GSEA) analysis to explore the possible mechanism associated with BRCA metastasis. The gene expression profile for GSEA analysis was obtained from the GSE175692 dataset, which included 184 BRCA metastasis samples. All the patients were divided into high and low expression groups according to the median gene expression level. Then, the potential pathway enriched in high/low expression groups was predicted by GSEA analysis. The threshold for GSEA analysis was set as follows: the number of permutations (1000), enrichment statistic (weighted), metric for ranking genes (Pearson).

2.6 Statistical analysis

The expression difference between the 2 groups was compared with independent samples T-test or Mann-Whitney test, and results were presented with Violin-chart and Box-scatter which contained the expression median, upper quartile, lower quartile, maximum and minimum. The effects of gene expression on the survival of patients were evaluated through survival analysis and Cox regression

showing hazard ratio (HR) and 95% confidence interval (CI). $P < 0.05$ was considered as the statistical significance.

3. Results

3.1 DEGs identification and function analysis

The GSE175692 dataset was used to filter the DEGs between BRCA samples with or without bone metastasis. A total of 74 significant DEGs were found according to absolute $\text{LogFC} > 1$ and $P\text{-value} < 0.05$, and the top 10 up-regulated and 10 down-regulated DEGs were marked (**Fig.1A**). KEGG analysis showed that 74 DEGs were mainly associated with infectious disease, signaling molecules and interaction (**Fig.1B**). The enriched pathway of partial DEGs was shown in **Fig.1C**.

Functional enrichment analysis was then performed to reveal the role of 74 DEGs in cancer progression (**Fig.2**). The significant enriched KEGG pathway included ECM-receptor interaction, Cytokine-cytokine receptor interaction and Human papillomavirus infection. For cellular component, the DEGs were largely located at the collagen-containing extracellular matrix and extracellular matrix. For biological process, DEGs primarily participated in extracellular structure organization. In terms of molecular function, DEGs were significantly enriched in receptor regulator activity and receptor ligand activity.

3.2 PPI network and significant hub genes analysis

The protein-protein interaction (PPI) network among DEGs was constructed using String and Cytoscape. The PPI network contained 60 nodes and 167 edges (**Fig.3**). Using the Degree gene ranking method, we identified the top 10 hub genes, namely Estrogen Receptor 1 (ESR1), Matrix Metalloproteinase 9 (MMP9), Bone Morphogenetic Protein 2 (BMP2), Secreted Phosphoprotein 1 (SPP1), C-C Motif Chemokine Ligand 2 (CCL2), Progesterone Receptor (PGR), Wnt Family Member 2 (WNT2), Matrix Metalloproteinase 3 (MMP3), Integrin Subunit Beta 3 (ITGB3), Prominin 1 (PROM1).

The consistent genes between the top 20 DEGs and 10 hub genes were determined to filter more significant hub genes. The 5 most significant hub genes were subsequently found which contained 3 up-regulated (MMP9, ITGB3 and BMP2) and 2 down-regulated (CCL2 and PROM1) DEGs (**Fig.4A**). The detailed information of 5 hub genes in GSE175692 was presented in **Fig.4B**. Differential expression of 5 hub genes between bone metastasis and non bone metastasis BRCA samples were then compared, and results of quantity analysis were shown in **Fig.4C**.

Differential expression of 5 hub genes between BRCA bone metastasis and non-bone metastasis samples was also verified using the GSE14020 dataset. The result showed that the comparison result of MMP9, ITGB3, BMP2 and PROM1 in GSE14020 conformed to that in GSE175692 (**Fig.5**). However, no difference was observed in terms of CCL2 mRNA expression in GSE14020.

We then explored the expression of 5 hub genes between normal and BRCA samples. The results showed that mRNA expression of PROM1, CCL2 and BMP2 were down-regulated in BRCA samples compared with

that in the normal group (**Fig.6A**, all FDR<0.05). No difference was observed between normal and BRCA groups in terms of ITGB3 and MMP9 mRNA expression. The protein expression of MMP9, ITGB3 and PROM1 were all downregulated in BRCA compared with that in the normal group (**Fig.6B**, all P<0.05). It should be noted that CCL2 and BMP2 were not identified in Breast data set.

3.3 Prognosis analysis on hub genes

We firstly performed the univariate cox regression to screen the prognosis-related biomarkers in metastatic BRCA and found that PROM1 and CCL2 showed a significant correlation with the overall survival of patients (**Fig.7A**). Further survival analysis indicated that high expression of PROM1 and CCL2 shortened the overall survival time of metastatic BRCA patients (**Fig.7B**).

Subsequently, the prognostic performance of PROM1 and CCL2 in metastatic BRCA was evaluated. A nomogram analysis showed that PROM1 possessed the largest contribution to the survival probability of patients, contributing 100 points (**Fig.8A**). The ROC analysis indicated that the prediction ability of PROM1 was slightly superior to CCL2 (**Fig.8B**).

According to the gene expression levels and clinical outcome, all the patients with metastatic BRCA were divided into high-risk and low-risk groups. The detailed information for risk score was presented in **Fig.9A**. For each individual, the risk score= $0.076806616 \times (\text{expression of PROM1}) + 0.12554159 \times (\text{expression of CCL2})$. Further, we found that the high-risk group showed a poor prognosis than the low-risk group (**Fig.9B**). The ROC curve presented the prediction ability of risk score on the survival probability (**Fig.9C**). The nomogram indicated that risk score possessed the best prognostic performance than PROM1 and CCL2 (**Fig.9D**). These results showed that the risk score model possessed favorable prediction ability on the survival probability of metastatic BRCA patients.

The above results indicated the significance of PROM1 and CCL2 in metastatic BRCA, we further verified their prognostic impacts using the GSE124647 dataset, which contained 140 samples with metastatic BRCA. The survival analysis in **Fig.10** showed that PROM1 still presented a significant prognostic impact. However, we found no impact of CCL2 on the survival time of metastatic BRCA patients.

The PROM1 and CCL2 exerted a significant effect on the survival of metastatic BRCA patients, we also explored their prognostic impacts on the survival of BRCA patients with single bone metastasis. However, both PROM1 and CCL2 exerted no significant impacts on the survival of BRCA patients with single bone metastasis (**Fig.11**), which might be due to the insufficient sample size.

We also used the GSE11121 dataset to verify the prognostic impact of PROM1 on the distant metastasis-free survival of BRCA samples. The best cutoff point of PROM1 expression for survival analysis was presented in **Fig.12A**. Survival analysis showed that PROM1 high expression was not conducive to the distant metastasis-free survival of BRCA patients (**Fig.12B**, P<0.001), indicating the correlation of PROM1 expression with distant metastasis in BRCA.

3.5 GSEA analysis on PROM1

PROM1 was determined as a vital down-regulated gene in bone metastasis and played an important role in metastasis BRCA progression. Finally, we explored the potential mechanism of PROM1 involved in metastasis BRCA. The GSEA analysis indicated that PROM1 was negatively associated with IGF1 and mTOR pathways (**Fig.13**). We speculated that PROM1 might influence metastasis BRCA development through negatively regulating IGF1 and mTOR pathways.

Discussion

It has been reported that 70% of patients with metastatic BRCA have a marked tendency to spread to the bone, resulting in significant skeletal complications and mortality [11]. Despite advances in diagnosis, the identification of patients at high risk of bone recurrence is still an unmet clinical need. Therefore, identifying useful biomarkers was conducive to improve the clinical outcome of metastatic BRCA patients. Spadazzi C et al. have identified TFF1 as strictly correlated to bone metastasis from ER+ breast cancer, and TFF1 upregulation could be useful to identify patients at high risk of bone metastasis [12]. Pantano F et al. determined ITGA5 as a predictive of poor bone metastasis-free survival [8]. As bone is the most frequent organ for breast cancer metastasis, and thus it is essential to predict the bone metastasis of breast cancer.

In this study, we firstly identified 74 significant DEGs between BRCA samples with or without bone metastasis. These 74 DEGs mainly participated in ECM-receptor interaction and Cytokine- cytokine receptor interaction, which referred to signaling molecules and interaction. The previous study has indicated that ECM-receptor interaction significantly participated in breast cancer metastasis to bone [13] and brain [14]. ECM-receptor interaction pathway was also correlated to lung metastasis in osteosarcomas [15], lung adenocarcinoma metastasis [16] and liver metastasis of colorectal cancer [17]. In addition, virus infection such as human papillomavirus (HPV) infection was also proved to correlated with DEGs in our study. During the last decades, great interest has been given to the viral etiology of breast cancer. Habyarimana et al. showed that HPV DNA was found in 46.81% of Rwandese breast cancer cases, HPV16 being the most prevalent subtype (77.27%) followed by HPV33 (13.64%) and HPV31 (9.09%) [18], suggesting high-risk HPV infections as a risk factor in breast cancer development. Cavalcante et al. found that the high frequency of HPV infection in breast cancer samples indicated a potential role in breast carcinogenesis [19]. However, Hedau et al. found no evidence of HPV etiology of breast cancer in Indian women [20]. And the low frequency of HPV was detected in Ghaffari et al. study, which also did not support the association between breast carcinoma and HPV infection [21]. It followed that effect of HPV infection in BRCA did not be uniform, and it was possible that HPV may be responsible for breast carcinogenesis only in a small percentage of all breast cancer.

Subsequently, we found 5 more important hub genes among all GEGs, namely MMP9, ITGB3, BMP2, CCL2 and PROM1. A univariate cox regression initially identified that PROM1 and CCL2 expressions were related to the prognosis of metastatic BRCA patients. Survival analysis, nomogram, and ROC curve analyses further presented the significance of PROM1 on the survival probability in metastatic BRCA. Expression analysis showed that PROM1 was upregulated in BRCA bone metastasis samples compared

with no bone metastasis samples. The above results showed that PROM1 was an important DEGs involved in bone metastasis of BRCA, and presented a good prediction performance on the patient's survival probability.

Prominin-1 (PROM1) is a transmembrane glycoprotein, which is expressed in stem cell lineages [22]. Katoh et al. determined the PROM1 as a functional cancer stem cells (CSC) marker [23]. At present, there were few kinds of research reporting the role of PROM1 in BRCA. Priedigkeit et al. reported that PROM1 was related to transcriptional remodeling in long-term estrogen-deprived locoregional breast cancer recurrences [24]. Bertheau et al. identified PROM1 as a mutant TP53-associated gene involved in breast cancer [25]. The important role of PROM1 in BRCA needs more investigation in the future. This study has indicated the important function of PROM1 in BRCA metastasis, and we finally explored the potential mechanism associated with PROM1. GSEA analysis indicated that PROM1 was significantly related to mTOR and IGF1 pathways with a negative correlation. The mTOR critically regulated several essential biological functions, such as cell growth, metabolism, survival, and immune response [26]. However, the mTOR was frequently deregulated in human cancers [27]. It has demonstrated that PROM1 played a key role in the regulation of autophagy via upstream suppression of mTOR signaling in the human retinal pigment epithelium [22]. Kholodenko et al. found that cells with the complete knockout of PROM1 showed the highest resistance to mTOR inhibitors in colorectal cancer [28]. Our study suggested that low expression of PROM1 might activate the mTOR pathway in BRCA metastasis samples. Insulin-like growth factors (IGF) are the most abundant growth factors in bone and are required for normal skeletal development and function. Via activation of the IGF-1 receptors (IGF-1R) and variant insulin receptors, IGFs promote cancer progression, aggressiveness, and treatment resistance [29]. Preclinical evidence has suggested that a high IGF-1 environment in primary tumor stimulated tumor cells metastasis to bone, suggesting that bone metastases may reflect IGF dependency [29]. Several studies have indicated that IGF signaling systems were able to regulate BRCA growth, progression, and metastasis [30, 31]. It followed that the IGF1 pathway played a vital function in the progression of BRCA metastasis. However, this study just indicated the negative correlation between PROM1 and IGF1/mTOR pathways, the detailed regulation in BRCA metastasis especially bone metastasis was worth further investigating.

Conclusion

This study identified 74 DEGs between BRCA samples with or without bone metastasis, and 5 important DEGs were then filtered, namely MMP9, ITGB3, BMP2, CCL2 and PROM1. Through Cox regression and Kaplan-Meier survival analysis, PROM1 and CCL2 were determined as the significant prognosis-related biomarkers associated with metastatic BRCA. And the risk model constructed by these 2 genes showed a favorable prediction property on patient's survival. Prognosis verification analysis further indicated the importance of PROM1 rather than CCL2. Especially, both nomogram and ROC analyses presented the better prediction ability of PROM1 on the survival probability of metastatic BRCA patients. Finally, we found that PROM1 negatively correlated with IGF1 and mTOR pathways involved in BRCA metastasis. This study identified PROM1 as an important prognosis-related biomarker associated with metastatic BRCA, and detailed function needed further investigation via experimental verification and clinical cohort.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

Funding: This work was supported by the National Natural Science Foundation of China (81802628) and Zhejiang Provincial Natural Science Foundation of China (LQ20H060007).

Authors' contributions: YW and CY analyzed, designed and interpreted the data about the breast invasive carcinoma. SZ and KH contributed to the discussion and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgments: Not applicable.

References

1. Chaffer CL, Weinberg RA: **A perspective on cancer cell metastasis.** *Science* 2011, **331**:1559-1564.
2. James JJ, Evans AJ, Pinder SE, Gutteridge E, Cheung KL, Chan S, Robertson JF: **Bone metastases from breast carcinoma: histopathological - radiological correlations and prognostic features.** *Br J Cancer* 2003, **89**:660-665.
3. Diessner J, Wischnewsky M, Stuber T, Stein R, Krockenberger M, Hausler S, Janni W, Kreienberg R, Blettner M, Schwentner L, et al: **Evaluation of clinical parameters influencing the development of bone metastasis in breast cancer.** *BMC Cancer* 2016, **16**:307.
4. Bado I, Gugala Z, Fuqua SAW, Zhang XH: **Estrogen receptors in breast and bone: from virtue of remodeling to vileness of metastasis.** *Oncogene* 2017, **36**:4527-4537.
5. Molnar IA, Molnar BA, Vizkeleti L, Fekete K, Tamas J, Deak P, Szundi C, Szekely B, Moldvay J, Vari-Kakas S, et al: **Breast carcinoma subtypes show different patterns of metastatic behavior.** *Virchows Arch* 2017, **470**:275-283.
6. Lee SJ, Park S, Ahn HK, Yi JH, Cho EY, Sun JM, Lee JE, Nam SJ, Yang JH, Park YH, et al: **Implications of bone-only metastases in breast cancer: favorable preference with excellent outcomes of hormone receptor positive breast cancer.** *Cancer Res Treat* 2011, **43**:89-95.

7. Awolaran O, Brooks SA, Lavender V: **Breast cancer osteomimicry and its role in bone specific metastasis; an integrative, systematic review of preclinical evidence.** *Breast* 2016, **30**:156-171.
8. Pantano F, Croset M, Driouch K, Bednarz-Knoll N, Iuliani M, Ribelli G, Bonnelye E, Wikman H, Geraci S, Bonin F, et al: **Integrin alpha5 in human breast cancer is a mediator of bone metastasis and a therapeutic target for the treatment of osteolytic lesions.** *Oncogene* 2021, **40**:1284-1299.
9. Westbrook JA, Wood SL, Cairns DA, McMahon K, Gahlaut R, Thygesen H, Shires M, Roberts S, Marshall H, Oliva MR, et al: **Identification and validation of DOCK4 as a potential biomarker for risk of bone metastasis development in patients with early breast cancer.** *J Pathol* 2019, **247**:381-391.
10. Zhang X, Yu X, Zhao Z, Yuan Z, Ma P, Ye Z, Guo L, Xu S, Xu L, Liu T, et al: **MicroRNA-429 inhibits bone metastasis in breast cancer by regulating CrkL and MMP-9.** *Bone* 2020, **130**:115139.
11. Xu Y, Zhang S, Liao X, Li M, Chen S, Li X, Wu X, Yang M, Tang M, Hu Y, et al: **Circular RNA circKBKB promotes breast cancer bone metastasis through sustaining NF-kappaB/bone remodeling factors signaling.** *Mol Cancer* 2021, **20**:98.
12. Spadazzi C, Mercatali L, Esposito M, Wei Y, Liverani C, De Vita A, Miserocchi G, Carretta E, Zanoni M, Cocchi C, et al: **Trefoil factor-1 upregulation in estrogen-receptor positive breast cancer correlates with an increased risk of bone metastasis.** *Bone* 2021, **144**:115775.
13. Chen X, Pei Z, Peng H, Zheng Z: **Exploring the molecular mechanism associated with breast cancer bone metastasis using bioinformatic analysis and microarray genetic interaction network.** *Medicine (Baltimore)* 2018, **97**:e12032.
14. Tang D, Zhao X, Zhang L, Wang Z, Wang C: **Identification of hub genes to regulate breast cancer metastasis to brain by bioinformatics analyses.** *J Cell Biochem* 2019, **120**:9522-9531.
15. Liu D, Zhou R, Zhou A: **Identification of key biomarkers and functional pathways in osteosarcomas with lung metastasis: Evidence from bioinformatics analysis.** *Medicine (Baltimore)* 2021, **100**:e24471.
16. Wang Y, Shi M, Yang N, Zhou X, Xu L: **GPR115 Contributes to Lung Adenocarcinoma Metastasis Associated With LAMC2 and Predicts a Poor Prognosis.** *Front Oncol* 2020, **10**:577530.
17. Machackova T, Vychytilova-Faltejskova P, Souckova K, Trachtova K, Brchnelova D, Svoboda M, Kiss I, Prochazka V, Kala Z, Slaby O: **MiR-215-5p Reduces Liver Metastasis in an Experimental Model of Colorectal Cancer through Regulation of ECM-Receptor Interactions and Focal Adhesion.** *Cancers (Basel)* 2020, **12**.
18. Habyarimana T, Attaleb M, Mazarati JB, Bakri Y, El Mzibri M: **Detection of human papillomavirus DNA in tumors from Rwandese breast cancer patients.** *Breast Cancer* 2018, **25**:127-133.

19. Cavalcante JR, Pinheiro LGP, Almeida PRC, Ferreira MVP, Cruz GA, Campelo TA, Silva CS, Lima L, Oliveira BMK, Lima LM, et al: **Association of breast cancer with human papillomavirus (HPV) infection in Northeast Brazil: molecular evidence.** *Clinics (Sao Paulo)* 2018, **73**:e465.
20. Hedau S, Kumar U, Hussain S, Shukla S, Pande S, Jain N, Tyagi A, Deshpande T, Bhat D, Mir MM, et al: **Breast cancer and human papillomavirus infection: no evidence of HPV etiology of breast cancer in Indian women.** *BMC Cancer* 2011, **11**:27.
21. Ghaffari H, Nafissi N, Hashemi-Bahremani M, Alebouyeh MR, Tavakoli A, Javanmard D, Bokharaei-Salim F, Mortazavi HS, Monavari SH: **Molecular prevalence of human papillomavirus infection among Iranian women with breast cancer.** *Breast Dis* 2018, **37**:207-213.
22. Bhattacharya S, Yin J, Winborn CS, Zhang Q, Yue J, Chaum E: **Prominin-1 Is a Novel Regulator of Autophagy in the Human Retinal Pigment Epithelium.** *Invest Ophthalmol Vis Sci* 2017, **58**:2366-2387.
23. Katoh M: **Canonical and non-canonical WNT signaling in cancer stem cells and their niches: Cellular heterogeneity, omics reprogramming, targeted therapy and tumor plasticity (Review).** *Int J Oncol* 2017, **51**:1357-1369.
24. Friedigkeit N, Ding K, Horne W, Kolls JK, Du T, Lucas PC, Blohmer JU, Denkert C, Machleidt A, Ingold-Heppner B, et al: **Acquired mutations and transcriptional remodeling in long-term estrogen-deprived locoregional breast cancer recurrences.** *Breast Cancer Res* 2021, **23**:1.
25. Bertheau P, Turpin E, Rickman DS, Espie M, de Reynies A, Feugeas JP, Plassa LF, Soliman H, Varna M, de Roquancourt A, et al: **Exquisite sensitivity of TP53 mutant and basal breast cancers to a dose-dense epirubicin-cyclophosphamide regimen.** *PLoS Med* 2007, **4**:e90.
26. Sun SY: **mTOR-targeted cancer therapy: great target but disappointing clinical outcomes, why?** *Front Med* 2021, **15**:221-231.
27. Murugan AK: **mTOR: Role in cancer, metastasis and drug resistance.** *Semin Cancer Biol* 2019, **59**:92-111.
28. Kholodenko IV, Kim YS, Gisina AM, Lupatov AY, Kholodenko RV, Yarygin KN: **Analysis of the Correlation between CD133 Expression on Human Colorectal Adenocarcinoma Cells HT-29 and Their Resistance to Chemotherapeutic Drugs.** *Bull Exp Biol Med* 2021, **171**:156-163.
29. Rieunier G, Wu X, Macaulay VM, Lee AV, Weyer-Czernilofsky U, Bogenrieder T: **Bad to the Bone: The Role of the Insulin-Like Growth Factor Axis in Osseous Metastasis.** *Clin Cancer Res* 2019, **25**:3479-3485.
30. Papadakis GZ, Mavroudis D, Georgoulas V, Souglakos J, Alegakis AK, Samonis G, Bagci U, Makrigiannakis A, Zoras O: **Serum IGF-1, IGFBP-3 levels and circulating tumor cells (CTCs) in early breast cancer patients.** *Growth Horm IGF Res* 2017, **33**:28-34.

31. Karlikova M, Topolcan O, Narsanska A, Kucera R, Treskova I, Treska V: **Circulating Growth and Angiogenic Factors and Lymph Node Status in Early-stage Breast Cancer - A Pilot Study.** *Anticancer Res* 2016, **36**:4209-4214.

Figures

Differential expressed genes (DEGs) identification and mechanism exploration. (A) Volcano plot and top 20 DEGs presentation in GSE175692. (B) KEGG pathway and classification. (C) Correlation between DEGs and KEGG pathway. Abbreviation: DEGs, differential expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

KEGG

Biological process

Cellular component

Molecular function

Figure 2

Functional enrichment analysis on 74 DEGs containing KEGG pathway and GO annotation. Abbreviation: DEGs, differential expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

Figure 3

The PPI network among DEGs. Green nodes indicated the top 10 hub genes identified by the Degree method. Abbreviation: DEGs, differential expressed genes; PPI, protein-protein interaction network.

Figure 4

Significant hub genes determination. (A) Venn analysis for determining 5 hub genes. (B) The detailed information of 5 hub genes in GSE175692. (C) The mRNA expression of 5 hub genes in BRCA samples with or without bone metastasis in the GSE175692 dataset. Abbreviation: BRCA, breast cancer.

Figure 5

The mRNA expression verification of 5 hub genes between BRCA bone metastasis and non-bone metastasis samples in GSE14020 dataset. Abbreviation: BRCA, breast cancer.

Figure 6

The differential expression of 5 hub genes between normal and BRCA samples. (A) The mRNA expression analysis is based on TCGA data. (B) Protein expression analysis based on CPTAC data. CCL2 and BMP2 were not identified in Breast data set. Abbreviation: BRCA, breast cancer.

Figure 7

The prognostic impact of 5 hub genes on the overall survival of metastatic BRCA samples in GSE175692. (A) Cox regression. (B) Kaplan-Meier survival analysis. Abbreviation: BRCA, breast cancer.

Figure 8

prognostic performance analysis on PROM1 and CCL2 in metastatic BRCA in GSE175692. (A) Nomogram. (B) ROC curve. Abbreviation: BRCA, breast cancer; ROC, receiver operating characteristic; AUC, area under curve.

Figure 9

The risk score analysis on PROM1 and CCL2 in GSE175692. (A) The risk score of patients and gene expression level. (B) Kaplan-Meier analysis on the risk score. (C) ROC curve analysis on the risk score. (D) Nomogram. Abbreviation: ROC, receiver operating characteristic; AUC, area under curve.

Figure 10

The prognostic impact verification of PROM1 and CCL2 in GSE124647.

Figure 11

The prognostic impact of PROM1 and CCL2 on BRCA patients with single bone metastasis in GSE175692. Abbreviation: BRCA, breast cancer.

Figure 12

The prognostic impact of PROM1 mRNA expression on the distant metastasis-free survival of BRCA patients using GSE11121. (A) The best cutoff point of expression level. (B) Survival analysis on PROM1. Abbreviation: BRCA, breast cancer; HR: hazard ratio.

Figure 13

GESA analysis on PROM1 in metastasis BRCA samples using GSE175692 dataset. Abbreviation: BRCA, breast cancer; GSEA, gene set enrichment analysis.