Candidate biomarkers and gene modules investigation for bone tumor samples derived from castration-resistant prostate cancer bone metastasis patients using WGCNA

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Research article

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Abstract

Background About 80-90% of castration-resistant prostate cancer (CRPC) patients would develop bone metastasis, which leads to the disorder of bone metabolism and induces skeletal related events. However, except for the few approved radiotherapeutic and chemotherapy drugs, like radium-223 and denosumab, there is still lack of effective treatment targeting the bone metastatic tumor. It is necessary and significant to explore the mechanisms of bone metastasis and tumorigenesis, especially the differences between the tumor and normal cells in bone after metastatic colonization, which will provide a set of candidate genes for the screening of novel bone targeting agents.

Results 4 datasets (GSE32269, GSE101607, GSE29650 and GSE74685) were obtained from the GEO database. 1983 differentially expressed genes (DEGs) were first identified between bone marrow tumor samples and normal marrow samples in GSE32269, followed by the weighted gene co-expression analysis. Most of the top 10 DEGs are found to be related with prostate cancer. 7 co-expression modules were then detected based on the 1469 DEGs shared by the 4 datasets, and 3 of them were found highly preserved among the other three datasets. The top 30 hub genes of the 3 modules were extracted. Among the enriched pathways of preserved modules, Cell adhesion molecules (CAMs) and Leukocyte transendothelial migration might play significant important roles in the tumor development in bone marrow. Literature searches further showed that a set of DEGs and hub genes might also contribute to the development of tumor in bone.

Conclusions Together, our findings not only provide outline of expression profile in CRPC bone metastasis, but also screen a set of genes associated with CPRC tumor cell colonization and development of bone tumor, which could be helpful for novel bone targeting agents screening.

Background

Prostate cancer (PCa) is one of the most common cancers and the tenth most common cause of cancer related mortality in men in China[1]. The rankings rise first in men in the developed countries[2]. Castration-resistant prostate cancer (CRPC) is an advanced form of prostate cancer by disease progression following surgical or pharmaceutical castration. This process is not inevitable, which is usually companied by poor prognosis and reduced survival time. To be known, CRPC patients are also at high risk of developing metastases. The common sites are bone, lymph nodes, liver, lungs and brain. However, bone is the most prominent site for metastases. About 80–90% of CRPC patients develop bone metastases[3]. Bone metastases could lead to the disorder of bone metabolism and induce skeletal related events(SREs), such as pathological fracture, spinal cord compression and hypercalcemia, which not only reduce survival time and life quality, but also increase burden of treatment[4].

At present, the diagnose of CRPC bone metastases was mainly based on Symptoms, Imaging and Histopathology. The treatment of CRPC bone metastases was mainly divided into three categories[5]: first, Radiotherapeutic drug, like radium–223, which was approved to treat the mCRPC patients; second,

bone targeting therapy, like Denosumab, which was also approved to treat the patients with solid tumor bone metastases; third, trial therapy, like Cabozantinib and Dasatinib. Among them, the bone targeting agents showed a significantlly and potentially clinical actionability. However, the development of novel bone targeting agents needs a deep understanding of mechanisms of bone metastasis and tumorigenesis. At present, the widely accepted mechanism of bone metastasis is the ‘seed and soil hypothesis’, which describes an interaction between circulating tumor cell and microenvironment of bone tissue[6]. Most of further researches focus on dissecting the process of initiation to development of distant metastasis, such as cancer cells migrate through the endothelial cells to gain access to systemic circulation via the tortuous and leaky tumor vasculature and cell signaling aberrations[7, 8]. However, these researches do not explore the state of tumor cells after metastatic colonization and also do not detect the differences between the tumor cells and normal cells in bone, which could be more helpful to screen novel bone targeted agents. Therefore, based on the mentioned consideration, this study selected multiple expression datasets of bone marrow tumor samples derived from CRPC bone metastases patients, and hope to identify some candidate modules and genes through differential expression analysis and weighted gene co-expression network analysis. These modules and genes represent the significant changes of expression profiles in bone tumor cells, which will provide a cluster of candidates for further agents screening and validation.

Materials And Methods

Data collection and preprocessing

Four expression profile datasets containing CRPC bone metastasis were downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo). Dataset GSE32269 was chosen for further analysis with 29 CRPC bone metastatic marrow samples and 4 normal bone marrow samples, which was used for bone cancer significantly genes selection and correlated modules detection. The other three datasets GSE101607, GSE29650 and GSE74685 were kept with only CRPC bone metastatic samples, which was used to validate and screen the truly significant and preserved bone cancer related modules. Detailed information of datasets was shown in table 1.

Before the analysis, all the raw data were reprocessed. Probes were mapped to gene symbols. Empty probes and probes mapping to multiple genes were both discarded according to each annotation platform. If there were multiple probes that mapped to the same gene symbol, their mean values were considered as the gene expression value. The reprocessed data was normalized by the limma (linear models for microarray data) package in R[9].

Identification of differentially expressed genes (DEGs)

The eBayes analysis was used to detect the DEGs between bone metastatic marrow samples and bone normal marrow samples in GSE32269 using limma package[9]. The adjusted P-value <0.05 and |log-fold

change|>1 were set as the threshold for DEGs screening.

Enrichment Analysis

R package clusterProfiler[10] was used for the Enrichment analysis of genes. False discovery rate (FDR) <0.05 was set as the threshold for the identification of significant GO-Enrichment terms and Pathway-Enrichment terms.

WGCNA analysis

The co-expression network analysis was performed using weighted gene co-expression network analysis (WGCNA) [11]. First, the soft threshold for network construction was selected, which is the lowest power for which the scale-free topology fit index curve flattens out upon reaching a high value. Second, the function blockwiseModules was used for one-step network construction and module detection. Then, the module eigengene (ME) of each module was calculated, and the correlation between MEs was also calculated. Finally, the key node (hub gene) was determined by high intramodule connectivity of genes. According to the intromodule connectivity, the top 30 hub genes in modules were visualized using VisANT software[12].

In addition, network preservation at the module level was conducted between GSE322269 and the other three datasets using the function modulePreservation[13]. The comparability of two datasets is assessed by correlating measures of average gene expression and overall connectivity of two datasets. The higher the correlations of these properties, the better chance you will have of finding similarities between the two datasets at subsequent stages of analysis.

Results

DEG identification of bone marrow tissue for CRPC bone metastatic patients

In order to detect the transcriptomic differences between CRPC bone metastatic marrow samples and normal marrow samples, GSE32269 with 29 metastatic borrow samples and 4 normal borrow samples was selected and downloaded from GEO databases. Differentially expressed genes were identified directly using the limma package. 1983 DEGs were screened with the threshold of |logFC|>1 and p.adjust<0.05, as shown in Fig1A. There were 416 up-regulated genes and 525 down-regulated genes for bone metastatic marrow samples. The top 10 significantly expressed genes are KLK3, KRT18, EFNA1, SLC396A, PGLYRP1, MGAM, RHD, NKX3–1, GFI1, EPB42, which were functionally associated with prostate cancer or bone marrow. For example, KLK3 is a candidate marker for diagnosis and monitoring of prostate cancer[14]. EFNA1 plays an important role in angiogenesis and tumor neovascularization[15]. The expression profiles of these DEGs were showed as heatmap in Fig1B. Enrichment analysis was

further conducted. The result was shown in Fig1C-D. The most enriched GO terms are neutrophil and leukocyte -associated terms. The top5 pathway terms are Malaria, Leukocyte transendothelial migration, B cell receptor signaling pathway, phagosome and chemokine signaling pathway.

WGCNA analysis

Since the 4 datasets come from different platforms, we should ensure that the 4 datasets are comparable. First, we need to limit the analysis to genes that expressed among the datasets. The intersection was taken among the DEGs of GSE32269 and the genes of other three datasets. 1469 genes were selected, and the corresponding expression profiles of these genes in 4 datasets were then prepared. Second, the comparability of GSE32269 and other dataset was assessed by measuring the average gene expression and overall connectivity between two datasets (Fig 2). It's clear to see that the correlations are positive and the p-value are significant in all cases, which suggests that the datasets are comparable.

The 1469 genes were further investigated as input for hierarchical clustering using the function hclust. We found that the 29 samples mainly yielded two clusters (Fig3A). where GSM799490, GSM799516, GSM799492, GSM799512, GSM799513, GSM799515 and GSM799517 clustered in a clade, and the other 22 samples clustered in the other clade.

Prior to gene co-expression network detection, the analysis of network topology for various soft-thresholding powers was performed to obtain relative balanced scale independence and mean connectivity. As shown in Fig3B, power 7 was the lowest power for which the scale-free topology fit index reaches 0.85. Based on this power, 7 modules were generated as shown in Fig3C. The largest module was the turquoise module, which contained 585 genes, the smallest module was the black module containing 49 genes. Averagely, each module contained 183 genes. Then, we calculate and cluster the eigengenes of entire modules on their correlations to further quantify co-expression similarities (Fig4). The 7 modules also yielded two main clusters: green, blue and red module form a small branch, and the other modules form the other branch.

Enrichment analysis was further performed to detect biological significance of each modules as listed in table S2 (Additional files). We found that each module has great difference in GO terms with each other, except that yellow, turquoise and brown modules shared 4 terms: GO:0043312, GO:0002283, GO:0042119, GO:0002446. The 4 terms were all related with leukocyte mediated immunity. Great differences were also observed in pathway terms among each module. Red module presents no significantly enriched pathways. Yellow and brown module shared an enriched pathway term, named Osteoclast differentiation. Turquoise and brown module also shared an enriched pathway term, named Malaria. Yellow and turquoise module shared two pathways, which are Leukocyte transendothelial migration and natural killer cell mediated cytotoxicity. It's worth noting that turquoise enriched pathways contain a set of signaling pathways, which are reported to be related with tumor development, such as B cell receptor signaling pathway, chemokine signaling pathway, NF-kappa B signaling pathway, Fc epsilon RI signaling pathway and hematopoietic cell lineage. In the yellow enriched pathways, Cell adhesion

molecules are related with cancer invasion and metastasis. Osteoclast differentiation is related with bone development. The green module is enriched with cell cycle-associated pathways.

Module validation among the other 3 datasets

In order to detect whether these modules are preserved between the other three datasets, module preservation statistics were calculated using the function modulePreservation. We set the threshold Z>10 to screen the highly preserved modules. As a result, Green, yellow and turquoise module are determined as the preserved modules. Besides, hub genes were determined according to the network topological index. Top 30 hub genes were investigated from each module as showed in Fig5.

Discussion

Development of bone metastases is a key and usual event in the progression of CRPC, which could lead to disorders of bone metabolism and skeletal related events. The median survival form men with bone metastases CRPC is approximately 1.5 to 2 years. At present, bisphosphonate and denosumab are the standard therapeutic drug for bone metastasis, but the overall therapeutic effectiveness is limited. Therefore, the development of novel bone targeting agents is an imperious demand. The purpose of this study was to dissect the expression profile differences between the established metastatic bone marrow samples and normal bone marrow samples and then screened some differentially expressed genes, co-expression modules and module-hub genes, all of which might be candidate biomarkers for the diagnose and treatment of bone tumor.

First, the screened differentially expressed genes are related with prostate cancer or bone development or blood development. For example, among the top 10 up-regulated genes, KLK3 and KLK2, are highly enriched in prostate cancer, which are taken as effective biomarkers for diagnose and prognostic monitoring of prostate cancer[16]. GOLM1[17], FOLH1B[18], STEAP1[19] and PLPP1[20] are also identified as a candidate biomarker for prostate cancer. AGR2 expressed strongly in prostate tissue and show increased expression in prostate cancer[21]. Interestingly, there are two genes showing different results. ACCP gene acts as a tumor suppressor of prostate cancer through dephosphorylation of ERBB2 and deactivation of MAPK-mediated signaling. It should be expected to express lowly in the prostate cancer, including CRPC bone metastases. Similarly, decreased TSPAN1 was identified to promote prostate cancer progression[22]. However, both genes showed a highly expressed values with more than 16 fold change.

As for the top10 down-regulated genes, all of them are identified to be overexpressed in whole blood according to GTEx[23] and take part in embryonic development of blood and bone according to LifeMap Discovery[24]. However, further literature search results show no clear connection between bone tumor and these genes. Some of them were found to take an important in the tumorigenesis of other types of tumors. For example, Luo G et al. found that down-regulated LTF may serve as an important role in the dysregulation of the MAPK signaling pathway, which could induce the tumorigenesis of gastric tissue.

CEACAM1 was reported to be highly expressed in several different cancers and is correlated with tumor progression, metastasis and overall survival[25, 26]. The consistency and inconsistency with previous researches suggested that the tumorigenesis mechanism of bone metastasis of CRPC has changed compared to the tumorigenesis mechanism of primary prostate cancer. In a word, the genes showing inconsistent results might be significant features to detect and dissect tumorigenesis mechanisms of bone tumor.

Besides above DEGs, the modules identified among the DEGs using WGCNA analysis should get more attention, which predicts clusters of candidate genes involved in the tumorigenesis of bone marrow samples. Especially, the 3 modules (yellow, turquoise and green module), validated in other three datasets (GSE101607, GSE29650 and GSE74685), need further exploration. The top 30 hub genes were extracted from highly preserved modules and the corresponding expression profile were presented (Fig6). Surprisingly, all of these hub genes were all down-regulated in the bone marrow tumor samples. Pathway enrichment results of these hub genes were also showed. Compared to the enrichment results of whole module genes, three pathways were kept respectively in yellow and green module. Two of them should get more attention, which are Cell adhesion molecules (CAMs) and Leukocyte transendothelial migration. As fa as we know, CAMs take part in the process of integrating differentiation, proliferation and pro-survival signals from the surrounding microenvironment to the inner cell, which enables the numerous cell-cell and cell-matrix interactions within the bone marrow microenvironment and the controlled lifelong self-renewal and progeny of hematopoietic stem and progenitor cells[27]. Leukocyte transendothelial migration is generally activated in cancer progression, which hampers the anti-tumor responses of the host[28]. The involved hub genes of these two pathways are CTSS, CXCR4, HLA-A/E, ITGA4, NCF2, PTPRC and VCAM1. Literature searches show that low expression of them might lead to the dyregulation of the pathways, which could directly affect tumor development. For example, CXCR4, the G-protein-coupled receptor, could lead to chemotaxis, enhanced intracellular calcium, cell adhesion, survival, proliferation, and gene transcription. Abnormal expression of CXCR4 is reported to be related with tumor growth, invasion, angiogenesis, metastasis, relapse, and therapeutic resistance[29]. Down-regulation of cathepsin S (CTSS) was also identified to suppress triple-negative breast cancer growth and metastasis[30]. NCF2 encodes p67phox, the cytosolic subunit of the NADPH oxidase enzyme complex, which acts as a p53 target gene. Down-regulation of NCF2 could stimulates apoptosis[31]. In the bone marrow, cancer cell VCAM−1 attracts and tethers α4 integrin-expressing osteoclast progenitors to facilitate their maturation into multinucleated osteoclasts that mediate osteolytic metastasis. Aberrant expression of VCAM−1 mediates distinct tumor-stromal interactions in bone microenvironments[32].

In addition, some of other hub genes were also found to show correlation with tumor or bone development. For example, PRKCB, was reported as a disease-specific druggable target for treatment of Ewing sarcoma, and the loss of PRKCB could induce apoptosis in vitro and prevented tumor growth[33]. CCNB2[34] and RRM2[35], could promote carcinogenesis. Gas7[36, 37], TNFAIP2[38], CENPF[39], TOP2A[40], PTTG1[41], and CTSS[30], are reported to be overexpression in different cancers. Low expression or knockdown of these genes could lead to the suppression of tumor cell proliferation, metastasis and invasion.

In a word, this study has screened a set of candidate genes that might contribute to the tumorigenesis of bone marrow cells. But the expression levels of these genes presented an obvious difference in bone tumor compared to other types of tumors. This phenomenon suggested that tumor development in bone induced by CRPC bone metastases could have a different mechanism. The screened modules and hub genes would be good targets for further researches of tumorigenesis of bone and biomarkers screening.

Conclusion

In summary, this research creatively uses public data to identify tumor-related modules and genes based on transcriptional network analysis. Three modules were found to be highly preserved among 4 GEO datasets. Literature searches showed that some of them are involved in bone development or related with tumorigenesis. And some of the enriched hub genes are also implicated to be correlated with tumorigenesis in different types of cancers. Therefore, the hub genes of preserved modules might be used to offer clusters of candidate genes associated with the tumorigenesis of bone cancer. This might contribute to improving the understanding of mechanisms of cancer cell metastasis to the bone. However, more researches and experiments are needed to validate the roles of these genes in the process of tumorigenesis.

Abbreviations

CRPC: Castration-Resistant Prostate Cancer; DEG: Differentially Expressed Gene, WGCNA: Weighted Gene Correlation Network Analysis; ME: module eigengene

Declarations

Ethics approval and consent to participate

The data employed in this study are publicly available at: https://www.ncbi.nlm.nih.gov/geo. These genetic data have been employed in published studies and have been approved by the corresponding ethics committees.

Availability of data and materials

The data used in this study are available at: https://www.ncbi.nlm.nih.gov/geo. The accession numbers are GSE32269, GSE101607, GSE29650 and GSE74685.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

ZY and DY designed the study and drafted the manuscript. ZY, HZ and HW performed all the data analysis. HW helped the preparation of figures and tables. QL and DY contributed to the writing of the manuscript. All authors read and approved the final manuscript.

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Reference

1.Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J: *Cancer statistics in China, 2015. CA Cancer J Clin* 2016, *66(*2):115–132.

2.Siegel RL, Miller KD, Jemal A: *Cancer statistics, 2016. CA Cancer J Clin* 2016, *66(*1):7–30.

3.Wirth M, Tammela T, Cicalese V, Gomez Veiga F, Delaere K, Miller K, Tubaro A, Schulze M, Debruyne F, Huland H *et al: Prevention of bone metastases in patients with high-risk nonmetastatic prostate cancer*

treated with zoledronic acid: efficacy and safety results of the Zometa European Study (ZEUS). Eur Urol 2015, *67(*3):482–491.

4.Sathiakumar N, Delzell E, Morrisey MA, Falkson C, Yong M, Chia V, Blackburn J, Arora T, Kilgore ML: *Mortality following bone metastasis and skeletal-related events among men with prostate cancer: a population-based analysis of US Medicare beneficiaries, 1999–2006. Prostate Cancer Prostatic Dis* 2011, *14(*2):177–183.

5.Gdowski AS, Ranjan A, Vishwanatha JK: *Current concepts in bone metastasis, contemporary therapeutic strategies and ongoing clinical trials. J Exp Clin Canc Res* 2017, *36.*

6.Pedersen EA, Shiozawa Y, Pienta KJ, Taichman RS: *The prostate cancer bone marrow niche: more than just 'fertile soil'. Asian J Androl* 2012, *14(*3):423–427.

7.Wan LL, Pantel K, Kang YB: *Tumor metastasis: moving new biological insights into the clinic. Nat Med* 2013, *19(*11):1450–1464.

8.Weis SM, Cheresh DA: *Tumor angiogenesis: molecular pathways and therapeutic targets. Nat Med* 2011, *17(*11):1359–1370.

9.Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK: *limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res* 2015, *43(7*):e47.

10.Yu G, Wang LG, Han Y, He QY: *clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS* 2012, *16(*5):284–287.

11.Langfelder P, Horvath S: *WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics* 2008, *9:*559.

12.Hu ZJ, Chang YC, Wang Y, Huang CL, Liu Y, Tian F, Granger B, DeLisi C: *VisANT 4.0: Integrative network platform to connect genes, drugs, diseases and therapies. Nucleic Acids Research* 2013, *41(*W1):W225-W231.

13.Langfelder P, Luo R, Oldham MC, Horvath S: *Is my network module preserved and reproducible? PLoS Comput Biol* 2011, *7(*1):e1001057.

14.Penney KL, Schumacher FR, Kraft P, Mucci LA, Sesso HD, Ma J, Niu Y, Cheong JK, Hunter DJ, Stampfer MJ *et al: Association of KLK3 (PSA) genetic variants with prostate cancer risk and PSA levels. Carcinogenesis* 2011, *32(*6):853–859.

15.Dunne PD, Dasgupta S, Blayney JK, McArt DG, Redmond KL, Weir JA, Bradley CA, Sasazuki T, Shirasawa S, Wang T *et al: EphA2 Expression Is a Key Driver of Migration and Invasion and a Poor Prognostic Marker in Colorectal Cancer. Clin Cancer Res* 2016, *22(*1):230–242.

16.Fagerberg L, Hallstrom BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M, Tahmasebpoor S, Danielsson A, Edlund K *et al: Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics* 2014, *13(*2):397–406.

17.Varambally S, Laxman B, Mehra R, Cao Q, Dhanasekaran SM, Tomlins SA, Granger J, Vellaichamy A, Sreekumar A, Yu J *et al: Golgi protein GOLM1 is a tissue and urine biomarker of prostate cancer. Neoplasia* 2008, *10(*11):1285–1294.

18.Zhang Z, Wu H, Zhou H, Gu Y, Bai Y, Yu S, An R, Qi J: *Identification of potential key genes and high-frequency mutant genes in prostate cancer by using RNA-Seq data. Oncol Lett* 2018, *15(*4):4550–4556.

19.Gomes IM, Arinto P, Lopes C, Santos CR, Maia CJ: *STEAP1 is overexpressed in prostate cancer and prostatic intraepithelial neoplasia lesions, and it is positively associated with Gleason score. Urol Oncol* 2014, *32(*1):53 e23–59.

20.Uhlen M, Zhang C, Lee S, Sjostedt E, Fagerberg L, Bidkhori G, Benfeitas R, Arif M, Liu Z, Edfors F *et al: A pathology atlas of the human cancer transcriptome. Science* 2017, *357(*6352).

21.Maresh EL, Mah V, Alavi M, Horvath S, Bagryanova L, Liebeskind ES, Knutzen LA, Zhou Y, Chia D, Liu AY *et al: Differential expression of anterior gradient gene AGR2 in prostate cancer. BMC Cancer* 2010, *10:*680.

22.Xu F, Gao Y, Wang Y, Pan J, Sha J, Shao X, Kang X, Qin J, You MJ, Huang Y *et al: Decreased TSPAN1 promotes prostate cancer progression and is a marker for early biochemical recurrence after radical prostatectomy. Oncotarget* 2016, *7(*39):63294–63305.

23.Consortium GT: *The Genotype-Tissue Expression (GTEx) project. Nat Genet* 2013, *45(*6):580–585.

24.Edgar R, Mazor Y, Rinon A, Blumenthal J, Golan Y, Buzhor E, Livnat I, Ben-Ari S, Lieder I, Shitrit A *et al: LifeMap Discovery: the embryonic development, stem cells, and regenerative medicine research portal. PLoS One* 2013, *8(7*):e66629.

25.Dankner M, Gray-Owen SD, Huang YH, Blumberg RS, Beauchemin N: *CEACAM1 as a multi-purpose target for cancer immunotherapy. Oncoimmunology* 2017, *6(7*):e1328336.

26.Thies A, Moll I, Berger J, Wagener C, Brummer J, Schulze HJ, Brunner G, Schumacher U: *CEACAM1 expression in cutaneous malignant melanoma predicts the development of metastatic disease. J Clin Oncol* 2002, *20(*10):2530–2536.

27.Windisch R, Pirschtat N, Kellner C, Chen-Wichmann L, Lausen J, Humpe A, Krause DS, Wichmann C: *Oncogenic Deregulation of Cell Adhesion Molecules in Leukemia. Cancers* 2019, *11(*3).

28.Enarsson K, Lundin BS, Johnsson E, Brezicka T, Quiding-Jarbrink M: *CD4(+)CD25(high) regulatory T cells reduce T cell transendothelial migration in cancer patients. Eur J Immunol* 2007, *37(*1):282–291.

29.Chatterjee S, Behnam Azad B, Nimmagadda S: *The intricate role of CXCR4 in cancer. Adv Cancer Res* 2014, *124:*31–82.

30.Gautam J, Banskota S, Lee H, Lee YJ, Jeon YH, Kim JA, Jeong BS: *Down-regulation of cathepsin S and matrix metalloproteinase–9 via Src, a non-receptor tyrosine kinase, suppresses triple-negative breast cancer growth and metastasis. Exp Mol Med* 2018, *50.*

31.Italiano D, Lena AM, Melino G, Candi E: *Identification of NCF2/p67phox as a novel p53 target gene. Cell Cycle* 2012, *11(*24):4589–4596.

32.Lu X, Mu E, Wei Y, Riethdorf S, Yang QF, Yuan M, Yan J, Hua YL, Tiede BJ, Lu XM *et al: VCAM–1 Promotes Osteolytic Expansion of Indolent Bone Micrometastasis of Breast Cancer by Engaging alpha 4 beta 1-Positive Osteoclast Progenitors. Cancer Cell* 2011, *20(*6):701–714.

33.Surdez D, Benetkiewicz M, Perrin V, Han ZY, Pierron G, Ballet S, Lamoureux F, Redini F, Decouvelaere AV, Daudigeos-Dubus E *et al: Targeting the EWSR1-FLI1 Oncogene-Induced Protein Kinase PKC-beta Abolishes Ewing Sarcoma Growth. Cancer Res* 2012, *72(*17):4494–4503.

34.Lin SY, Pan HW, Liu SH, Jeng YM, Hu FC, Peng SY, Lai PL, Hsu HC: *ASPM is a novel marker for vascular invasion, early recurrence, and poor prognosis of hepatocellular carcinoma. Clin Cancer Res* 2008, *14(*15):4814–4820.

35.Wang N, Zhan T, Ke T, Huang X, Ke D, Wang Q, Li H: *Increased expression of RRM2 by human papillomavirus E7 oncoprotein promotes angiogenesis in cervical cancer. Brit J Cancer* 2014, *110(*4):1034–1044.

36.Chao CCK, Hung FC, Chao JJ: *Gas7 Is Required for Mesenchymal Stem Cell-Derived Bone Development. Stem Cells Int* 2013.

37.Hung FC, Chang YH, Sue LC, Chao CCK: *Gas7 Mediates the Differentiation of Human Bone Marrow-Derived Mesenchymal Stem Cells Into Functional Osteoblasts by Enhancing Runx2-Dependent Gene Expression. J Orthop Res* 2011, *29(*10):1528–1535.

38.Xie Y, Wang B: *Downregulation of TNFAIP2 suppresses proliferation and metastasis in esophageal squamous cell carcinoma through activation of the Wnt/beta-catenin signaling pathway. Oncol Rep* 2017, *37(*5):2920–2928.

39.Zhuo YJ, Xi M, Wan YP, Hua W, Liu YL, Wan S, Zhou YL, Luo HW, Wu SL, Zhong WD *et al: Enhanced expression of centromere protein F predicts clinical progression and prognosis in patients with prostate cancer. Int J Mol Med* 2015, *35(*4):966–972.

40.Jain M, Zhang LS, He M, Zhang YQ, Shen M, Kebebew E: *TOP2A is overexpressed and is a therapeutic target for adrenocortical carcinoma. Endocr-Relat Cancer* 2013, *20(*3):361–370.

41.Noll JE, Vandyke K, Hewett DR, Mrozik KM, Bala RJ, Williams SA, Kok CH, Zannettino ACW: *PTTG1 expression is associated with hyperproliferative disease and poor prognosis in multiple myeloma. J Hematol Oncol* 2015, *8.*

Table

Due to technical limitations, the table could not be displayed here. Please see the supplementary files section to access the table.

Additional Files

Additional file 1. Top 10 up- and down- regulated genes between metastatic bone tumor and normal samples in GSE32269.

Additional file 2. GO and KEGG enrichment analysis results for each module.

Figures

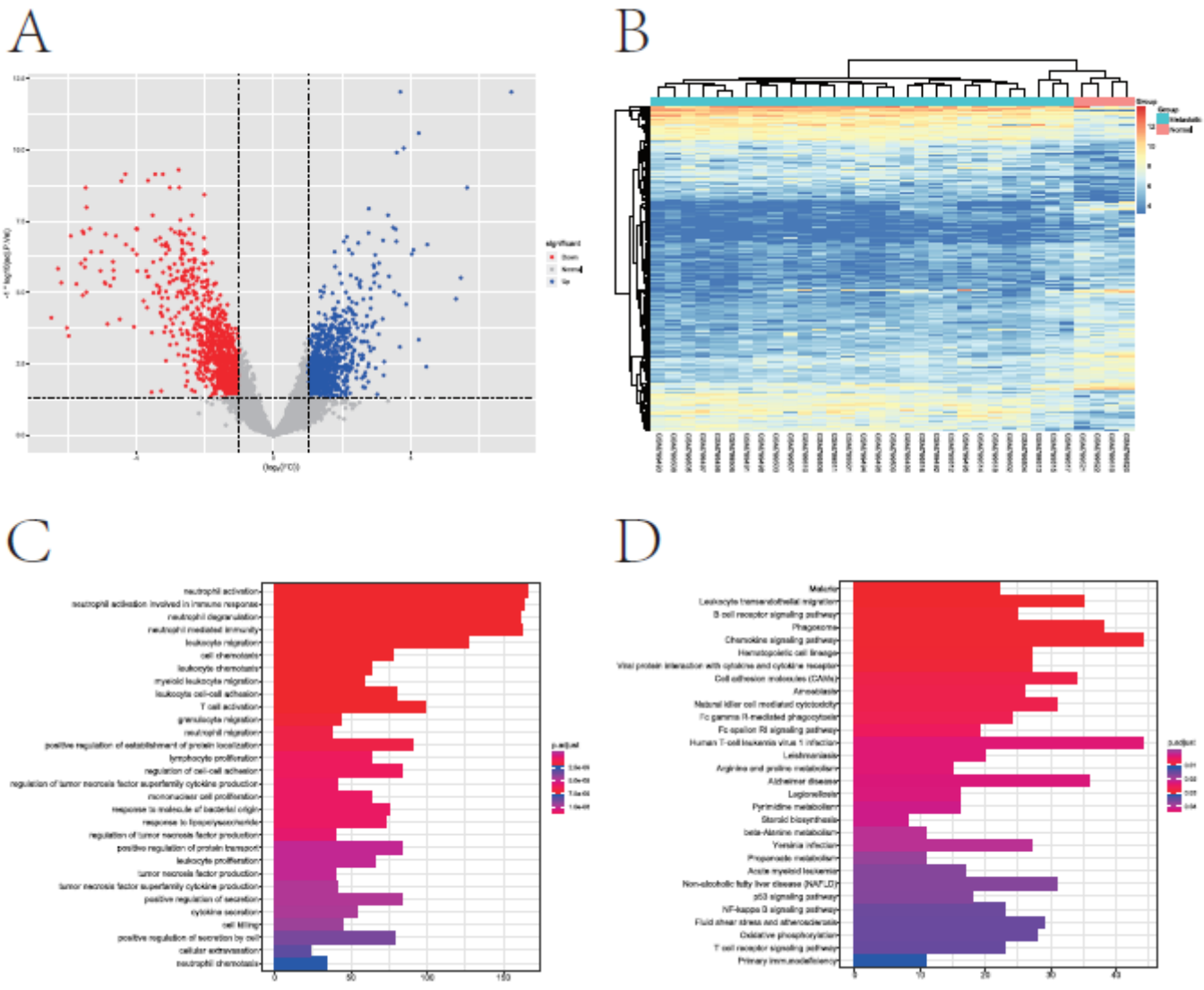

Figure 1

The volcano, heatmap, GO and KEGG enrichment results of differentially expressed genes (DEGs) between tumor and normal cells in bone. (A) the volcano plot for DEGs. Grey dots represent genes which are not differentially expressed, red dots represent the upregulated genes, and the blue dots represent the downregulated genes. (B) the heatmap for DEGs. (C) the annotation of gene ontology function of DEGs using GO enrichment analysis. (D) the annotation of pathway function of DEGs using KEGG enrichment analysis.

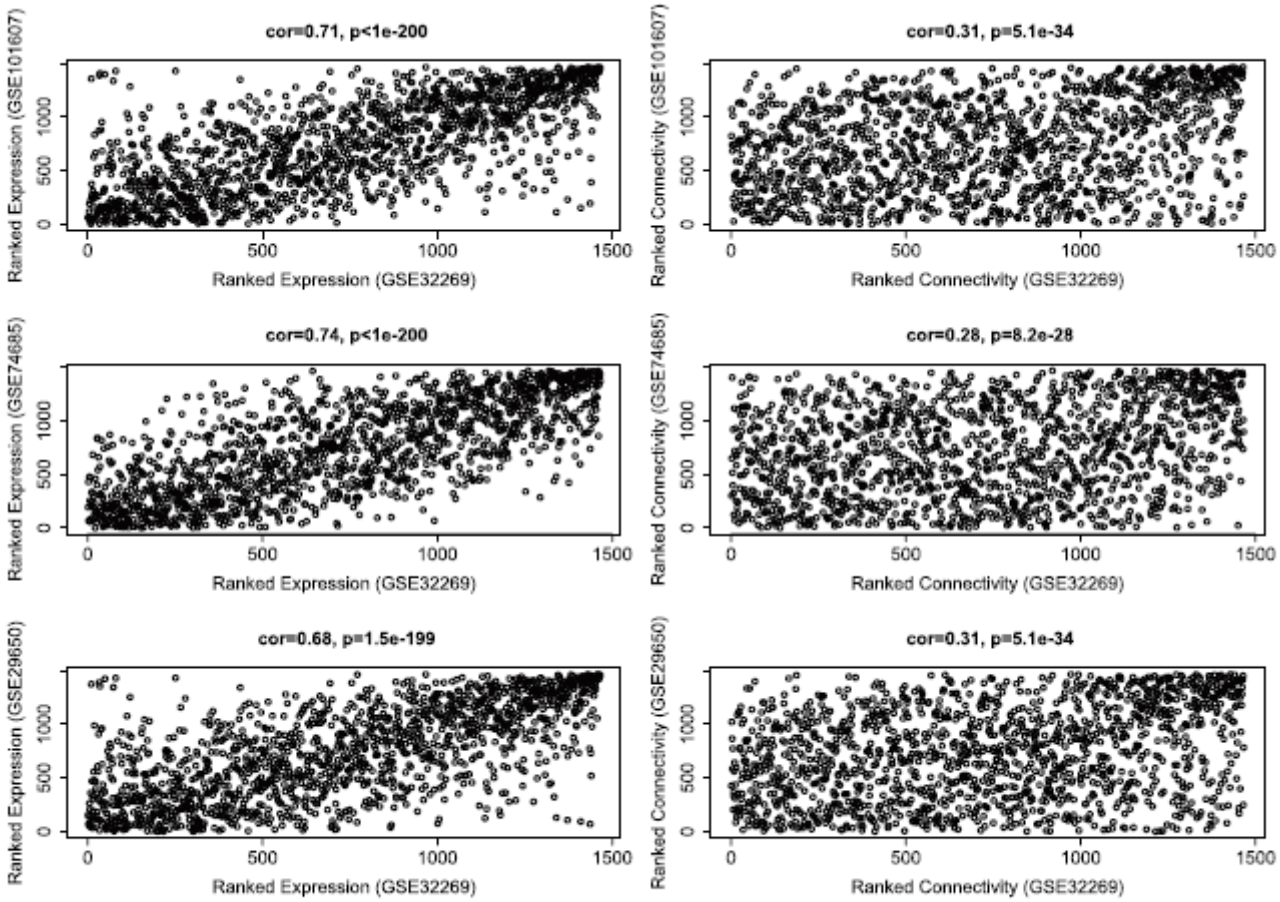

Figure 2

The correlations of average gene expression (Left) and overall connectivity (Right) between GSE32269 and other three datasets (GSE101607, GSE74685, GSE29650).

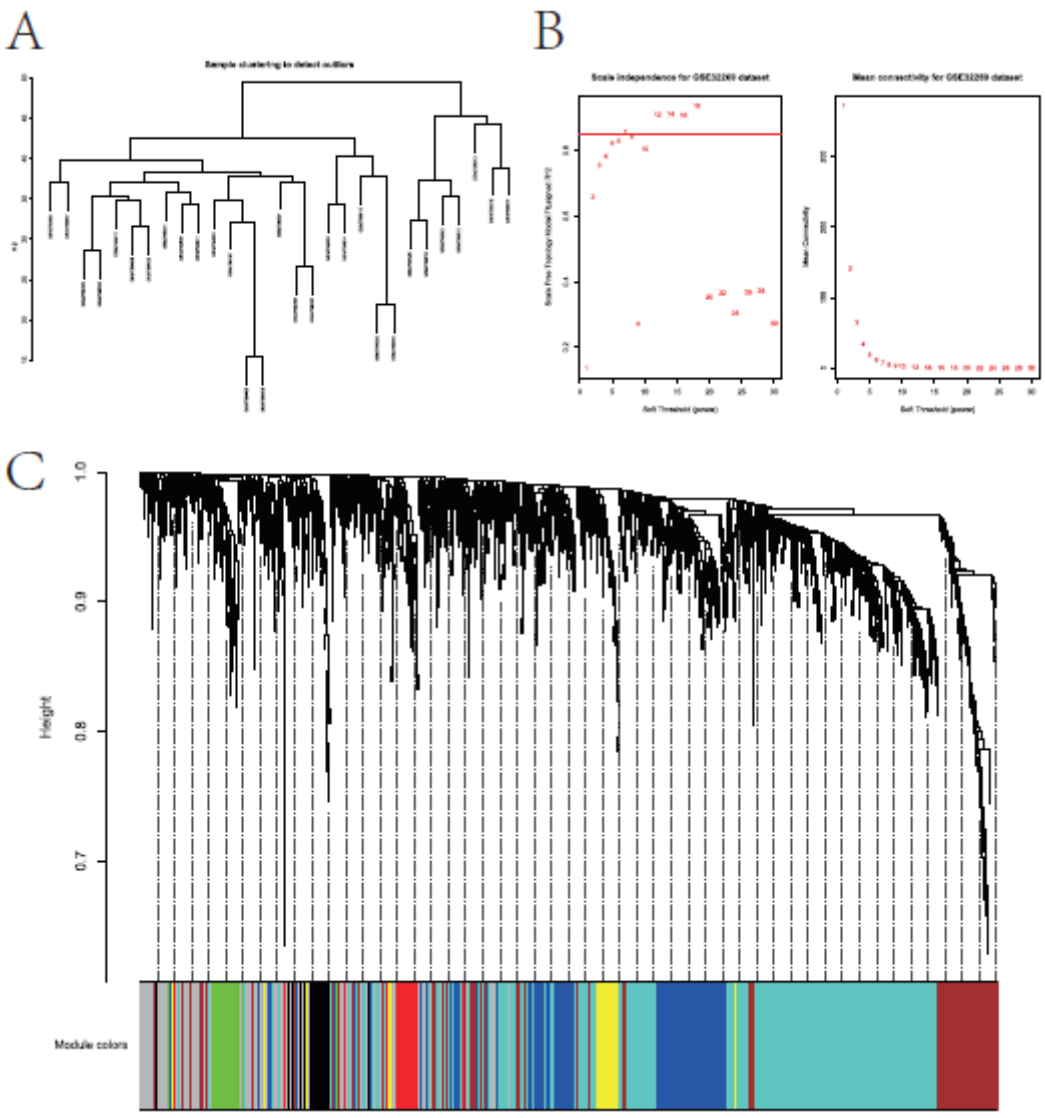

Figure 3

(A) Clustering dendrogram of samples based on DEGs in GSE32369. (B) Network topology of different soft-thresholding powers. The left panel displays the influence of soft-thresholding power (x-axis) on scale-free fit index (y-axis). The right panel shows the influence of soft-thresholding power (x-axis) on the mean connectivity (degree, y-axis). (C) Clustering dendrogram showing 8 modules that contain a group of highly connected genes. Each designated color represents a certain gene module.

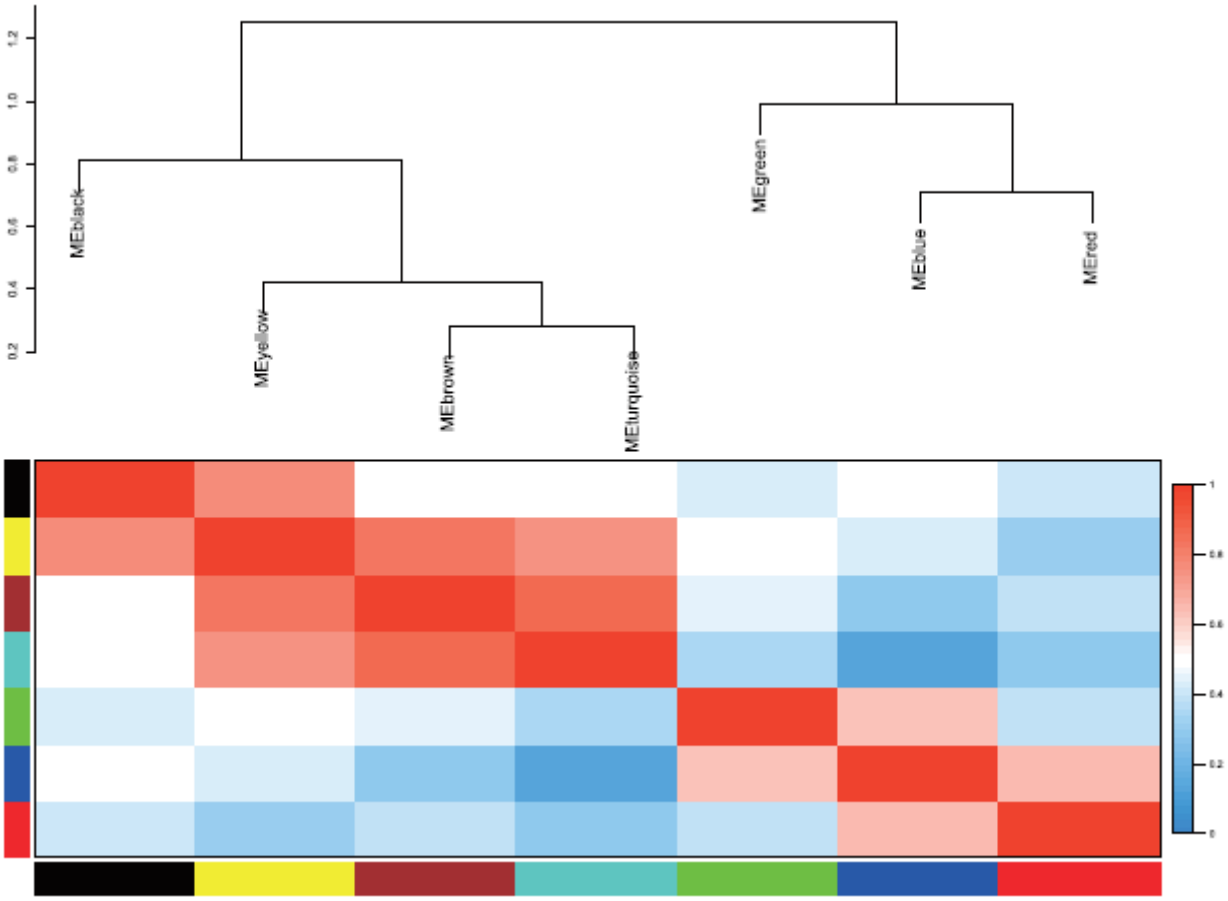

Figure 4

Hierarchical clustering of module genes in the clustering analysis (Upper) and heatmap plot of the adjacencies in the gene network (Lower). Each column and row correspond to one module. In the heatmap, red represents high adjacency (positive correlation) and blue represents low adjacency (negative correlation).

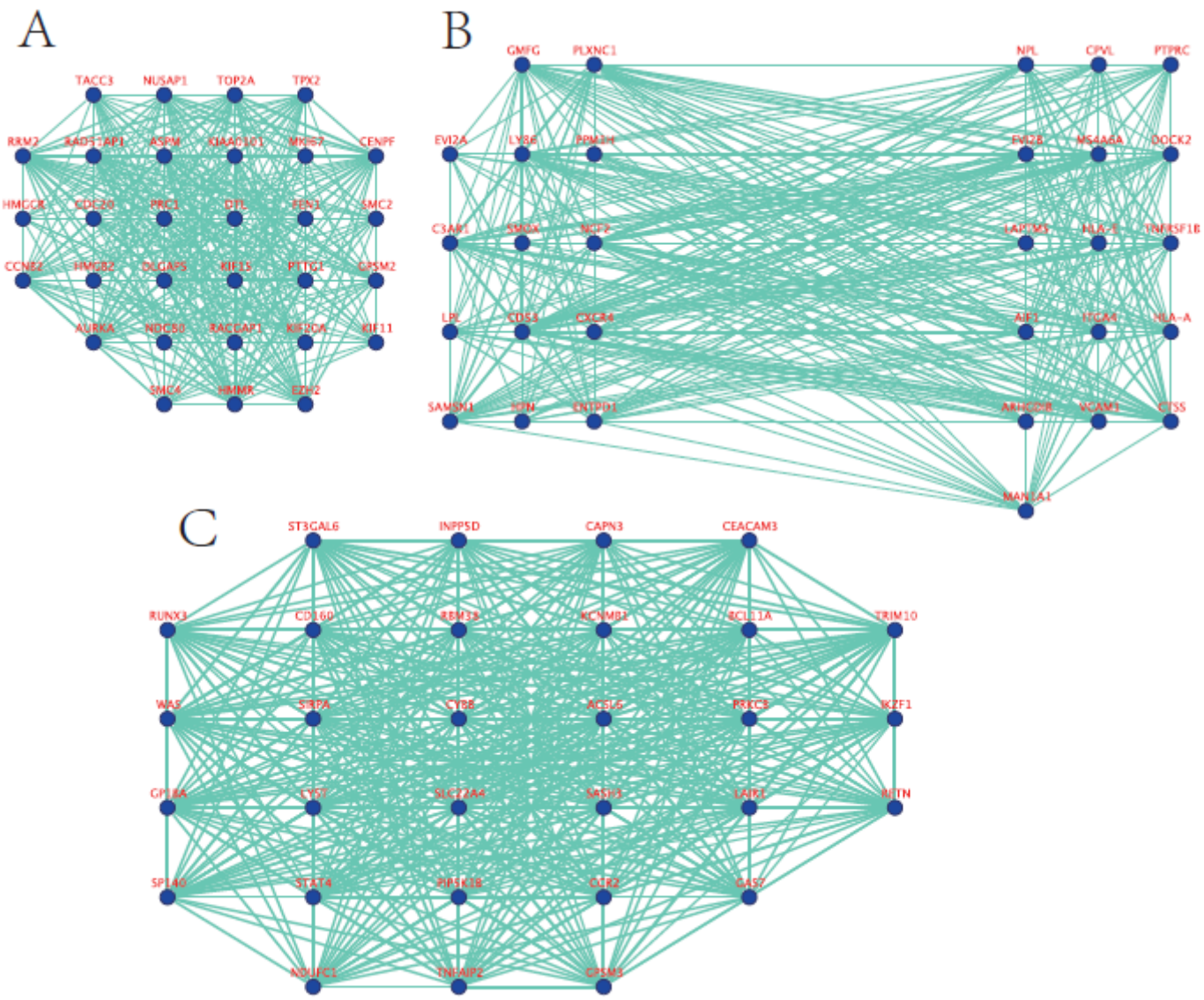

Figure 5

The visualization of green module (A), yellow module (B) and turquoise module (C).

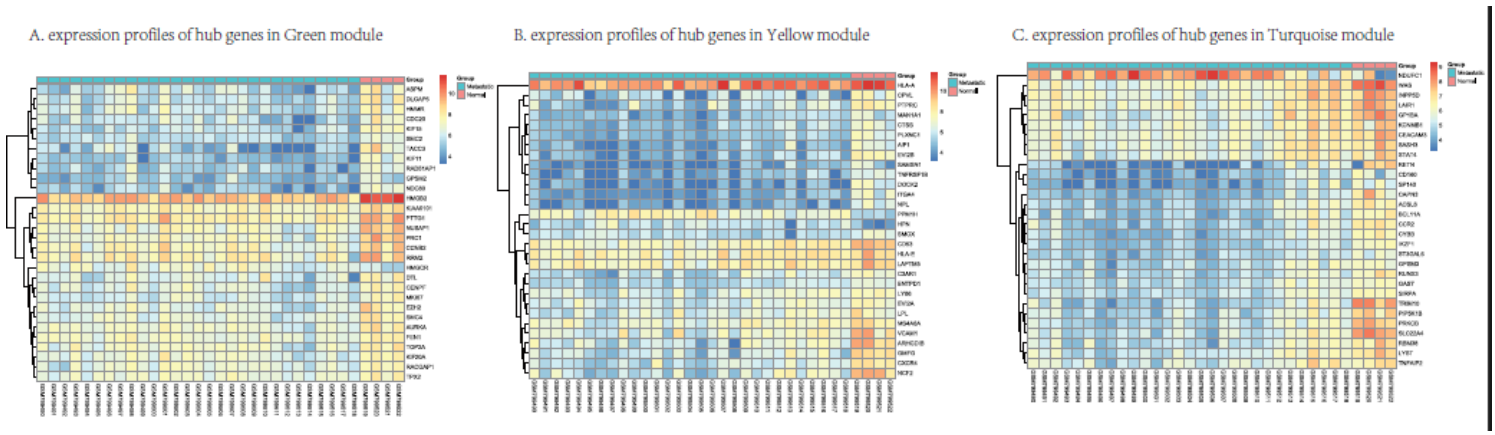

Figure 6

The expression heatmaps of hub genes in green (A), yellow (B) and turquoise (C) module between metastatic bone tumor and normal samples in GSE32269.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS2EnrichmentResultsforeachmodules.xlsx
- Table1Datasetsofgeneexpressionprofiles.xlsx
- TableS1Top10DEGsforupanddownregulatedgenes.xlsx