Copy Number Variation and Transcription Regulation of Genes and Immune Microenvironment of Tumor Affect the Stemness of Prostate Cancer Cell

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Research

Keywords: stemness of prostate cancer, WGCNA, ATAC-seq, CNV,immune infiltration

Posted Date: November 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-100256/v1

Abstract

Background: Prostate cancer stem cells (pCSC) play an important role in tumor metastasis through multiple pathways. From gene, protein to microenvironment, there are many factors affecting the cell stemness of prostate cancer (PCa). However, the effective factors affecting the stemness of prostate cancer cells are still unclear.

Methods: Based on the transcription data of prostate cancer in the TCGA database, WGCNA (Weighted Gene Co-expression Network Analysis) and stemness scores were used to find important stemness gene module. According to ATAC-seq and genomic data, analyze their relationship with stemness. The interaction of stemness genes was analyzed with STRING (functional protein association networks) database. Furthermore, based on the immune microenvironment score, the relationship between immune and stemness was analyzed.

Results: The most important stemness gene module in prostate cancer was obtained with WGCNA method; then a positive correlation between the gene CNVs (Copy Number Variants) of the most important stemness gene module and PCa stemness was found, as well as a positive correlation between the gene CNVs and Gleason score of PCa was also drug out. Further, the key transcriptional regulators of the most important stemness genes in PCa were obtained. In addition, it's found that immune cells, especially CD8+T cells and M1 macrophages, suppressed the stemness of PCa cells. Finally, by analyzing the protein interactions and the relationship between genes and immune cells, we found that interaction of the proteins of the most important stemness genes module and the relationship between these genes and immune cells of microenvironment of PCa were all important in affecting the stemness of PCa cells.

Conclusions: By analyzing multi-omics data of clinical specimen, we got the most important stemness genes and their important transcriptional regulators in PCa; and further mining analysis showed that the stemness of PCa cells is positive regulated by the CNVs and the interaction of the proteins of the most important stemness genes, and negatively regulated by the immune cells of the microenvironment of prostate cancer.

Background

Cancer stem cells (CSCs) are a few stemness-like cells with the ability of self-renewal and differentiation into cancer cells [1]. They play an important role in the occurrence and development of tumors, especially closely related to tumor metastasis [2-4]. In prostate cancer, the stemness of cancer cells (including prostate cancer stem cells, PCSC) are closely related to the metastasis of prostate cancer [5]. In the process of prostate cancer metastasis, PCSCs initiates EMT to form fibroblast-like cells, and then enter the blood. With the circulation system, prostate cancer cells migrate to other tissues (such as bone tissue and lymph tissue), and grow into tumor tissue in other tissues, which leads to tumor metastasis (cancer cell spreading).

As we known, many factors relate to stemness of CSC cells, not only including intracellular factors (such as stemness-related genes), but also including microenvironment of cancer tissues (such as immune cells in tumor microenvironment) [3, 6, 7]. In prostate cancer, it has been reported that the immune cells (especially CD8+ T cells and macrophages) in the microenvironment of prostate cancer are closely related to the metastasis of PCa cells [8, 9]. The number of immune cells around the early PCa tissue will decrease with the growth of the cancer tissue, which results in the immunity in the microenvironment of PCa also decrease [10]. With the development of PCa to the later stage (Gleason score to 6-10), some studies report that some immune cells (such as related T cells) in the microenvironment can reverse to promote or enhance the growth of PCa cells and help cancer cells metastasize [11].

Although increasing publications have reported the relationship between stemness and metastasis in PCa cells, there are few of studies on the stemness regulation of PCa cells [12, 13]. Lots of factors affecting the stemness of PCa cells remain unclear and need to investigate.

The bioinformatics method based on the TCGA database has been increasingly used to analyze the molecular basis of prostate cancer development and clinical patient prognosis [14-16]. By using appropriate analytical software and methods to analyze a variety of large size data of clinial specimen from the TCG database (including transcriptome sequencing data, gene sequencing data, ATAC-seq data, etc.), the molecular basis of prostate tumorigenesis, development of PCa and the prognosis of patients might be figure out [17, 18]. Therefore, the bioinformatics analysis of TCGA data should be helpful to provide some clues and direction for basic experimental research and clinical cancer treatment in PCa.

In this study, we obtained the most important stemness-related gene module in prostate cancer cells from transcriptome data and OCLR scores (for scoring the stemness of tumors). Further mining analysis showed that the cancer cell stemness in PCa tissues was positively correlated with the CNVs of the most important stemness-related genes and negatively correlated with the number of immune cells in the microenvironment of PCa tissue, and these correlations were all closely related to the clinical stage of PCa (such as Gleason score). Analysis results also demonstrated that some transcriptional regulators of the most important stemness-related genes were important in regulating the stemness of PCa cells. All our multi-omics analyzing results might provide some theoretical clues for us to experimentally investigate the factors affecting the PCa cell stemness and its relationships between PCSC and PCa metastasis.

Methods

Analysis of transcriptome (RNA-seq) data

Based on OCLR stemness scores and Gleason classification of TCGA prostate cancer clinical data, correlation analysis between PCa cell stemness and Gleason scores were carried out. By combing stemness scoreing, WGCNA [19] analysis was performed on the transcriptome data of prostate cancer in TCGA. Differential expression analysis of genes in the WGCNA results that were most relevant to PCa cell stemness was performed and presented in heatmap. Further, the transcriptome data of 33 samples from

GSE104786 (GEO database) were also analyzed for differential expression of stemness-related genes, and heatmap was drawn. In addition, the transcriptome data of SRR7651698, SRR7651699, SRR7651700, SRR7651715, SRR7651716, SRR7651717, SRR7651718, SRR7651719, SRR7651720 (SRA database) were used to analyze the difference of gene expression in prostate cancer lines and small cell carcinoma of prostate.

Analysis of gene CNV data

The genes most related to stemness in WGCNA results were screened and their locations in genome were obtained by local Perl script method. Combined with the CNV data of TCGA, the local Perl script was used to screen the segments containing the locations of stemness genes; and then by combined with Gleason score of TCGA prostate cancer samples, the CNV of stemness genes were calculated by GISTIC2.0 [20].

Analysis of ATAC-seq data

From TCGA database, we got the ATAC-seq data (including SRR7651660, SRR7651661, SRR7651662, SRR7651675 SRR7651676); and then 2kb data of upstream and downstream of the stemness genes were obtained by using Bowtie2 software [21]. According to the results, the TSS signal intensity of stemness genes was drown by using deeptools [22]. After analyzing the above alignment data using MACS [23] and HOMER [24], information about transcription regulators of the stemness genes were obtained; and the importance of each transcriptional factors were further obtained from TCGA transcriptome data by PCA analysis with R language. As an example, visualized the upstream transcriptional factor of EZH2 with Sushi [25] package in R language.

Analysis of tumor microenvironment and immune infiltration

The tumor microenvironment score and tumor immune infiltration score were calculated using ESTIMATE [26] and CIBERSORT [27], respectively. Then, the correlation between stemness score and immune score of PCa cells was calculated with R by combining with Gleason classification of prostate cancer. Based on CIBERSORT analysis results, the distribution map of immune cell components of different Gleason grades in prostate cancer were drawn with R, and the correlation network among immune cells was also drawn with R by according to the correlation and significance of different immune cells.

Protein interaction network and correlation between stemness genes and immune infiltration

After getting and analyzing PPI (Protein-Protein Interaction) data from the STRING (functional protein association networks) database, the PPI data of important stemness genes was obtained. And then interaction network of proteins of important stemness genes was drawn with Cytoscape [28]. In addition, GO and KEGG enrichment analysis of important stemness genes were carried out with ClusterProfiler [29]. and the results were combined with transcription data of important stemness genes and the results of immune infiltration to analyze and calculate the correlation between the important stemness genes and immune cells with R.

Results

Correlation analysis between cancer cell stemness and clinical grade in prostate cancer

Based on OCLR's results and transcriptome data of PCa clinical specimen in TCGA, the correlation between the stemness scores and Gleason grade was analyzed. The results showed that the PCa cell stemness was closely related to Gleason grade and increased with the increased of Gleason classification (Gleason scores; Fig. 1a). In addition, by using WGCNA (Weighted Gene Co-expression Network Analysis) to analyze the PCa transcriptome data for cancer cell stemness-related gene transcripts, we got 30 transcript modules which related to PCa cell stemness (Fig. 1b, Supplementary Fig.1a and 1b), and many stemness-related genes were included in each transcript module. About 88.2% of the 2158 known cell stemness-related genes [30] were found in the results of our WGCNA analysis (Supplementary Materials S1; Supplementary Fig. 1c). From the results, we also found that MEmagenta module had the most positive correlation with the stemness of PCa cells (Fig. 1b), suggesting that the genes in this module (Supplementary Table S1) might be significant and play key roles in the development of PCa.

From the analysis results, we found that the expression of genes in the MEmagenta module in PCa cancer specimen was generally higher than that in normal prostate specimen; and expression of these stemness gene in PCa increased with Gleason grade, and the highest expression level was found at Gleason score 5 (Fig. 1c). Further, we analyzed the data from 33 sample of GEO database and found that most of the stemness gene expression levels in small cell prostate cancer (SCPC) were higher than those in prostate adenocarcinoma (PRAD) (Supplementary Fig. 1D). Subsequently, the transcriptome data of different prostate (normal or PRAD) cell lines and SCPC cell lines were analyzed and it's found that the expression of stemness gene in PCa cells (PRAD cell lines) was higher than that in normal cells. while the expression level in SCPC cell lines was slightly higher than that in PRAD cell lines (Supplementary Fig. 1e).

In the genes of MEmagenta module (Supplementary Table S1), we found that the known genes closely related to cancer stemness in non-prostate cancer, such as BRCA1 [31], EZH2 [31], FOXM1 [32], CDC20 [33] and CDCA8 [34], were all clustered in this module, indicating these genes were also closely related to the stemness of prostate cancer cells. From supplementary table S1, we also found that EZH2 was the most significant gene in the correlation with stemness of PCa cells.

The CNVs of stemness-related genes were closely correlated to cell stemness and malignancy of prostate cancer

The segments containing all genes in the MEmagenta module were obtained from TCGA and then analyzed using GISTIC (Genome Identification of Significant Targets in Cancer) method. Analysis results showed that most of genes in MEmagenta module were found to have high variations, which were distinctly higher than those in normal prostate tissue, in prostate cancer, indicating that the stemness of PCa cells was influenced by the CNVs (Copy Number Variations) of these genes (Fig. 2a and

Supplementary Fig. 2a). In two types of CNV (amplification and deletion, Supplementary materials S2-S3), the change of deletion CNVs was more than amplification CNVs of the genes in MEmagenta module of PCa samples; and both amplification and deletion CNVs in PCa samples were all much more than those in prostate normal samples (Fig. 2b and 2c, and Supplementary Fig. 2b and 2c). In addition, by combining analysis with Gleason classification of PCa, GISTIC results showed that CNVs of genes in MEmagenta module were also increased with the Gleason socre (Fig. 2d), suggesting that CNVs of genes in MEmagenta module were related to the malignancy of PCa.

In details of CNVs of some genes in MEmagenta module, it was found that the CNVs (amplification) and expression of SKA3 and RUVBL1, which promoted tumor metastasis and played a role in the development of stem cells [35-38], were all increased with the clinical Gleason grade (Supplementary Fig. 3a and 3b); and the CNVs (deletion) and expression of MCM6 and CENPH, which enhanced cancer cell proliferation, stemness and metastasis and promoted cancer development [39-43], were also increased with the increase of clinical Gleason grade (Supplementary Fig. 3c and Fig. 3d).

Transcription regulation of stemness genes was correlated to stemness of PCa cells

ATAC-seq data of the MEmagenta module genes in PCa samples from TCGA, and the sequence data of 2kb range of the transcription start site (TSS) of the genes were analyzed and displayed. Results showed that both normal and tumor prostate samples had the binding signals of transcription factors, and the binding signals of all PCa samples (including different stage of PCa and small cell PCa) were weaker than that of normal prostate samples (Fig. 3a); and the binding signals of transcription regulators in small cell PCa was the weakest one (Fig. 3a, Supplementary Fig. 4b). These results indicated that the transcriptional regulation manner and the types of transcription regulators of the gene of MEmagenta moduel in PCa might be different with those in normal prostate tissues.

After clustering transcriptome of 253 common transcription factors in PCa, it was found that FOXA1, HOXB13, ERG1, et al. were highly expressed and NANOG, FOXA3, SOX3, et al. were lowly expressed in all prostate cancer (Supplementary Fig. 4a). In the top results of principal component analysis (PCA) of 253 common transcription factors, it was found that stemness-related PUM1 [44, 45], CLOCK [46], SP1 and TCF12 played a major positive regulation role on PCa cell stemness, while IRF3 [47] was a negative correlation with other 9 transcription factors and played negative regulation role on PCa cell stemness (Fig. 3b, Supplementary Fig. 4c). As we know, IRF3 (interferon regulatory factor 3) signaling played an essential role in TLR3-mediated apoptosis in LNCaP cells through the activation of the intrinsic and extrinsic apoptotic pathways [47], suggesting that the immune system might play a role in suppressing the stemness of PCa cells. Furthermore, out analysis results showed that AR, FOXA1, NFYA, CTCF and FOXO3 might enhance the stemness of PCa cells, where FOXF1 might be negatively correlated with these transcription factors (Fig. 3c).

In normal prostate samples, we found that the major transcription regulators were HOXB4, NFYB and TFE3 (Supplementary Fig. 5a and 5c), which were different from those in prostate cancer. In normal samples, the role of FOXA1, NFYA and FOXP1 in regulating stemness genes was changed, by comparing

their results of PCA analysis in prostate cancer (Supplementary Fig. 5b and Fig. 3c). These results indicated that same transcription factors might play different roles in regulating the cell stemness in prostate normal and cancer samples.

Our results of transcriptional regulator analysis showed that the upstream of EZH2 gene, the most relevant gene to the stemness of PCa cells, could be significantly bound by NFY (Fig. 3d). As a transcription factor, NFY not only regulates the self-renewal of hematopoietic stem cells [48], but also promotes the self-renewal and expansion of prostate cancer cells and their stemness [49]. Hence EZH2 might play a stemness roles in prostate cancer.

Immunological microenvironment negatively related to the stemness of PCa cells

By scoring the stemness and immunity of immune microenvironment of PCa in different Gleason stages of clinical samples, it was found there was a negative correlation between the PCa cell stemness and the immunity of microenvironment of PCa clinical samples in all stages; and the correlation coefficient of stage I-II was almost the same as that of stage III-V (Fig. 4a). By analyzing and scoring the stromal and immune cells of PCa microenvironment, we found that the scores of stromal and immune cells in PCa microenvironment were all inversely related to the stemness of PCa cells (Supplementary Fig. 6a); and immunity and immune cells of PCa microenvironment were improved and enhanced with PCa progression (Supplementary Fig. 6b). After clustering the genes of MEmangenta module based on cell stemness and immune scores of PCa, we found that PCa cell stemness was negatively related to immunity of PCa microenvironment in the clinical samples with high expression of genes of MEmagenta module (Supplementary Fig. 6d), indicating the immune microenvironment had an inhibitory effect on the cell stemness of PCa. Furthermore, by analyzing and scoring the immune infiltration of PCa clinical samples from TCGA, the number of 22 types of immune cells in prostate cancer with different clinical grades was obtained (Fig. 4b). The number of most types of immune cells in microenvironment of PCa was increased with the Gleason score increase; and CD8+ T cells [8] and macrophage M1 [9] were the most significantly increased in all types of immune cells, while the plasma cells [50] were significantly reduced with the Gleason score increase (Fig. 4b), indicating that plasma cells (B cells) in microenvironment of PCa played an important role in anti-PCa immunity. By analyzing the prostate tumor microenvironment immune cell network, it was found that the correlation among immune cells in cluster-A was more complicated than that in cluster-B, cluster-C and cluster-D (Fig. 4c; Supplementary Materials S4). For examples, the activated NK cell was negatively correlated with resting NK cell, which was most significant in all correlation among immune cells (Supplementary Fig. 6c) and the number of activated NK cell increased with the Gleason score increase in PCa (Fig. 4b), indicating that activated NK cell might play a major inhibitory funciton on prostate cancer stemness; the most significant positive correlation was between the activated dendritic cell and the memory B cell, and the number of both activated dendritic cells and memory B cells were all increased with the Gleason score increase (Fig. 4c), which indicated that these two type of cells might play important roles in inhibiting the stemness of PCa cells.

Protein interaction network of MEmagenta module genes and relationship between expression of MEmagenta module genes and immune infiltration of PCa

By screening and analyzing human protein interaction data containing MEmagenta module genes from STRING database, we not only found the important protein-protein interaction network in the proteins of MEmagenta module genes, but also found that EZH2 interacted directly with 17 proteins. In the EZH2-related 17 protein-protein interactions, we also found that EZH2 could regulate entire protein interaction network of MEmagenta module stemness genes by mainly interacting with CENPA, BUB1B and PARP1 (Fig. 5a) [51-54]. Furthermore, function enrichment analysis of genes of MEmagenta module revealed that most function of these genes were concentrated in cell mitosis, and the most significant functional pathway was related cell cycle (Supplementary Fig. 7a and 7b), suggesting that these stemness genes might involve in the regulation of PCa stem cell mitosis.

From the analysis results of relationship between expression of MEmagenta module gene and immune infiltration of PCa, we found that different immune cells had different effects on the expression of stemness genes in PCa; and different type of immune cells could have effects on the same stemness gene expression, as well as one stemness gene expression could also reversely affect different type of immune cells (Supplementary materials S5). In correlation of expression of stemness genes and immune cells, we found that expression of most stemness genes were positively correlated to memory B cells and naive B cells and negatively correlated to plasma cells (Fig .5b); and further, the number of these B cells increased, while the number of plasma cells decreased, with the increase of PCa Gleason score (Fig. 4b). These results indicated that B cells might play the opposite effects on PCa cell stemness in different conditions (it's consistent with reference [55]). In addition, the number of activated NK cells and memory CD4+ T cells were all increased with the Gleason score; and the expression of stemness genes of MEmagenta module was positively correlated to resting NK cells and memory CD4+ T cells, while negatively correlated to activated NK cells (Fig. 4b and 5b).

Expression of EZH2, the most relevant gene to PCa cell stemness, was most positively correlated to the activated memory CD4+ T cells and most negatively correlated to the resting Mast cells (Fig. 5c and 5d). Most types of cells positively correlated to the expression of EZH2 gene were T cells and B cells (Fig. 5c), suggesting that T cells and B cells were the important immune cells in regulating PCa cell stemness by controlling the expression of EZH2 gene. Further, the number of resting mast cells decreased and number of activated mast cell increased with the increased PCa Gleason scores (Fig. 4b); and the resting mast cells were the most negative correlated to expression of EZH2 gene (Fig. 5c and 5d). These results indicated that the immunity and immune cells of the microenvironment of PCa played an important role in the tumorigenesis and development of prostate cancer.

Discussion

It had been identified that cancer stem cells were closely related to cancer cell migration and played critical roles in tumor metastasis in PCa in clinic [56]. The research on the stemness of PCa cells and its

influencing factors were inevitably becoming a key direction in the field of tumor research. In this study, we found the gene module (MEmagenta module) most related to stemness of prostate cancer cells by analyzing multi-omics of PCa clinical samples and cell lines, and also found that the CNVs and transcriptional regulators of gene of MEmagenta module were the important influencing factors related to the stemness of prostate cancer cells by conjoint analysis of transcriptome data, gene CNV data and ATAC-seq data in prostate cancer. In the analysis results of immune microenvironment of prostate cancer, it's found that the immune microenvironment of PCa played a key role and negatively correlated with the stemness of prostate cancer cells, and the different immune cells in the immune microenvironment of PCa had different correlations with expression of stemness genes of MEmagenta module. It's said that the immune microenvironment of PCa and its immune cells were also the important influencing factors related to the stemness of PCa cells.

It's reported that tumor cell stemness could affect the immune response of tumor immune microenvironment and then result in tumor heterogeneity [30]. Although there had been many studies on the relationship between tumor cell stemness and tumor development in other tumors, there are few studies on the relationship between tumor cell stemness and PCa development, especially on the factors affecting and regulating the cell stemness of prostate cancer. In this study, based on a variety of omics data, by fully using a variety of bioinformatics analysis software and methods, we not only obtained two factors related to PCa cell stemness, including the CNVs of stemness-related genes and the immunity of tumor microenvironment, but also obtained the gene set most related to stemness of PCa cells. Many key genes included in this gene set and their transcription regulators were important in regulating and affecting PCa metastasis by incluencing PCa cell stemness. EZH2 not only promotes the formation of cancer stem cells, but also expand the aggressive cancer cells population and lead to cancer progression [57]. Furthermore, EZH2 can also co-regulate prostate cancer stem cell properties with BRCA1 [58]. RUBVL1 was a gene related to the stemness of prostate cancer cells, and its copy number increase with the tumor progresses. It is reported that RUVBL1 was essential for the survival of hematopoietic stem cells [38] and its gene copy number was increased in head and neck squamous cancers [59]. MYBL2 helped DNA double-strand repair in hematopoietic stem cells [60] and the emergence of CNV led to the occurrence and development of cancer [61, 62]. AURKB could determine the identity of embryonic stem cells [63] and the deletion of copy number also contributes the formation of aggressive tumors [64]. PUM1 regulated the expression of hematopoietic stem cells [65] and promoted the migration of cancer cells [45]. CLOCK regulated the biological clock of cancer stem cells and promoted the self-renewal of cancer cells [66]. HMGCS1 could promote cancer development [67] and affect the function of NK cells [68]. SUV39H1 attenuated the apoptosis of cancer cells [69] and enhanced the immune escape of tumor cells [70]. In addition to the reported genes related to cancer cell stemness, our results also showed many genes that had not been reported to be related to cancer cell stemness, especially to PCa cell stemness, such as TEDC2, TMEM132A and VARS etc.

In prostate cancer, up to now, there are few studies on the relationship between cell stemness regulatory factors and tumor malignancy. From the results of our multi-omics analysis, the CNVs of cancer cell stemness-related genes was closely related to the malignant degree of prostate cancer (Gleason score).

The CNVs of genes positively related to PCa cell stemness was also positively correlated with the degree of malignant of prostate cancer (Gleason scores); otherwise, the CNVs of genes negatively related to PCa cell stemness was negatively correlated with Gleason scores of prostate cancers. The correlations between transcriptional regulators of PCa cell stemness genes and prostate tumor malignancy were similar to the correlation between CNVs of PCa cell stemness genes and prostate tumor malignancy.

Although we found that the CNVs and transcriptional regulations of PCa cell stemness genes and the immune infiltration of prostate cancer were all important factors in influencing the stemness of PCa cells and further regulating the development of PCa and we also analyzed and obtained the key gene set and genes in regulating the stemness of PCa cells, the experimental evidence and the detailed mechanism of the correlation and regulation among CNVs of PCa cell stemness genes, transcription regulators of PCa cell stemness genes and immunity of PCa microenvironment were still unclear and needed to be further investigated in the future.

Conclusions

Our study finds that the cancer cell stemness in PCa tissues was positively correlated with the CNVs of the most important stemness-related genes and negatively correlated with the number of immune cells in the microenvironment of PCa tissue, and these correlations were all closely related to the clinical stage of PCa (such as Gleason score). Analysis results also demonstrated that some transcriptional regulators of the most important stemness-related genes were important in regulating the stemness of PCa cells. However, its detailed molecular mechanism needs future experimental verification in vivo and vitro.

List Of Abbreviations

pCSC: Prostate cancer stem cells; PCa: Prostate cancer; WGCNA: Weighted Gene Co-expression Network Analysis; STRING: functional protein association networks; CNVs: Copy Number Variants; CSCs: Cancer stem cells; PPI: Protein-Protein Interaction; SCPC: small cell prostate cancer; PRAD: prostate adenocarcinoma;

Declarations

Acknowledgements

Thanks to Dr. Wei Guanyun for her suggestions on data analysis.

Funding

This research was funded by the National Natural Science Foundation of Chia (NNSFC) (grant no. 81872104 and 81472415).

Authors’ contributions

Data analysis, writing original draft Zao Dai; Review and revised the manuscript Ping Liu.

Availability of data and materials

All data generated or analyzed during this study are available.

Consent for publication

Not applicable

Competing interests

The authors declare no conflict of interest.

Data statement

All data generated or analyzed during this study are included in this article.

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Figures

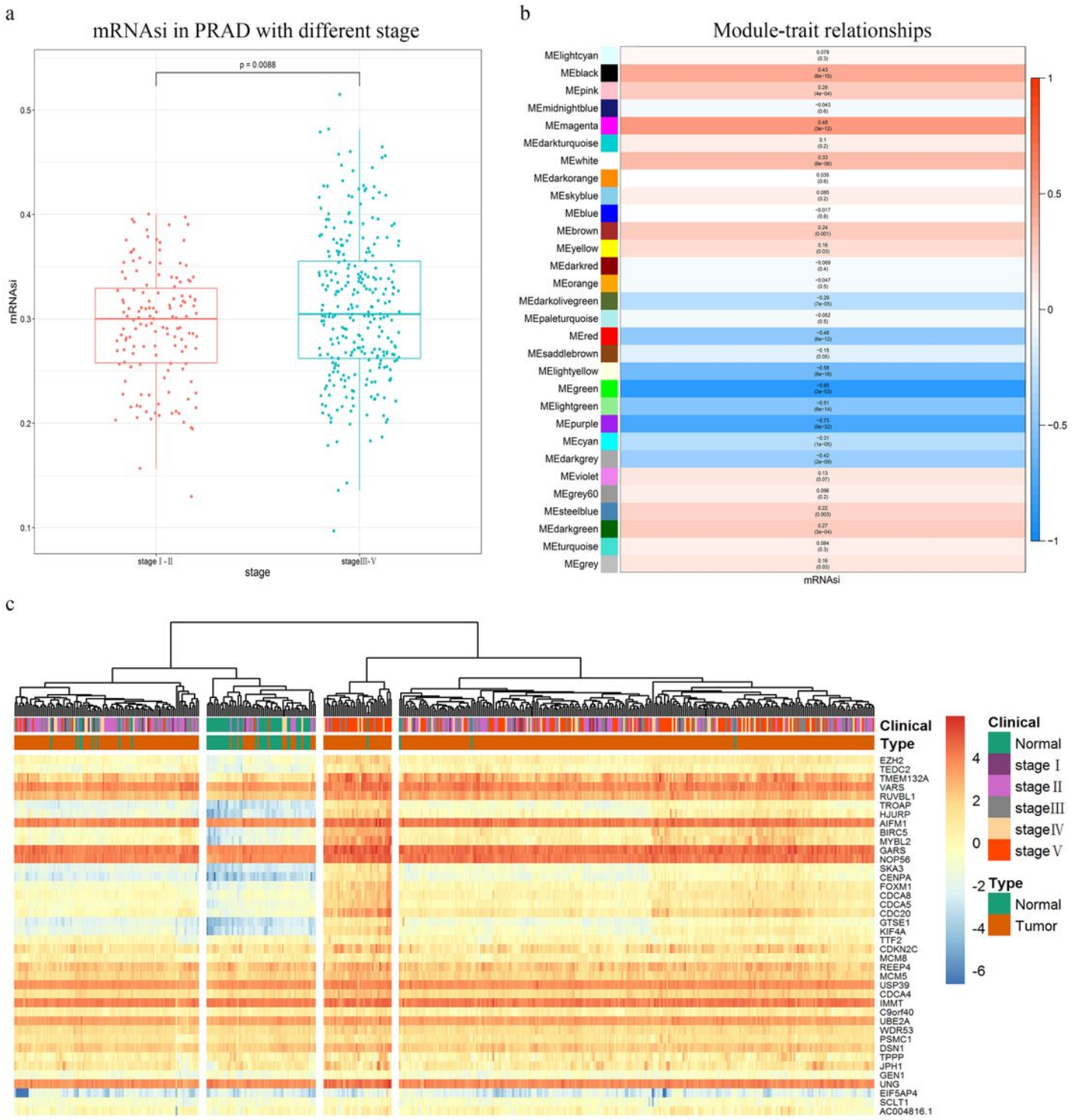

Figure 1

The relationship between stemness score and module and clinical grade a The relationship between prostate cancer stemness score and clinical grade. b The gene module related to stemness is based on the WGCNA method. Red represents a positive correlation between gene module and stemness, and blue represents a negative correlation between gene module and stemness. c The heatmap of genes in MEmagenta module, red represents high gene expression, blue represents low gene expression

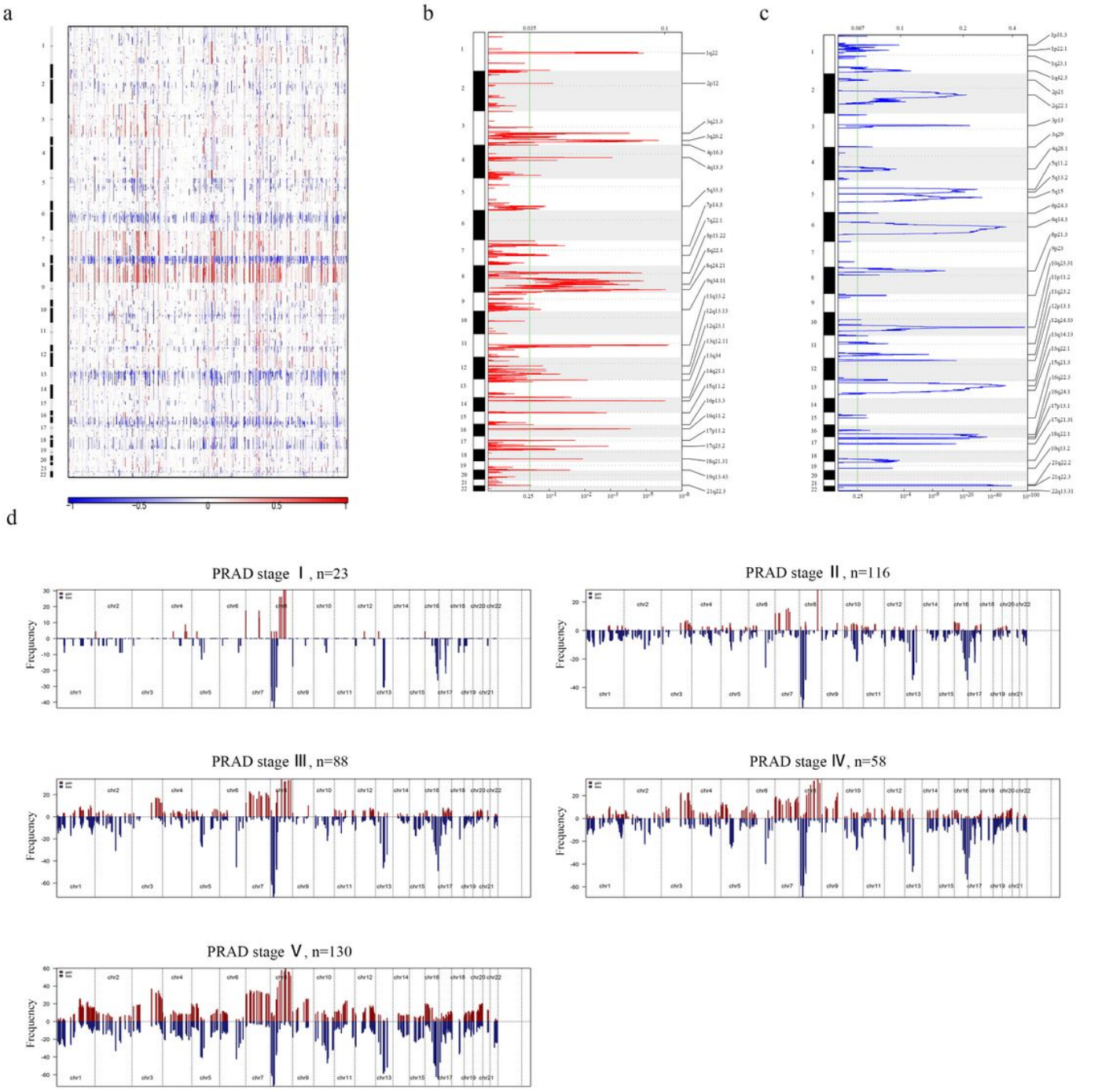

Figure 2

The stem gene CNV is relate to cell stemness and malignancy of prostate cancer a, b and c Changes of stem gene CNV in tumor samples, Red and blue represent the two types of CNV, amplification and deletion, respectively. d The stem gene CNV increases with the increase of clinical grade. Red and blue represent the two types of CNV, amplification and deletion, respectively.

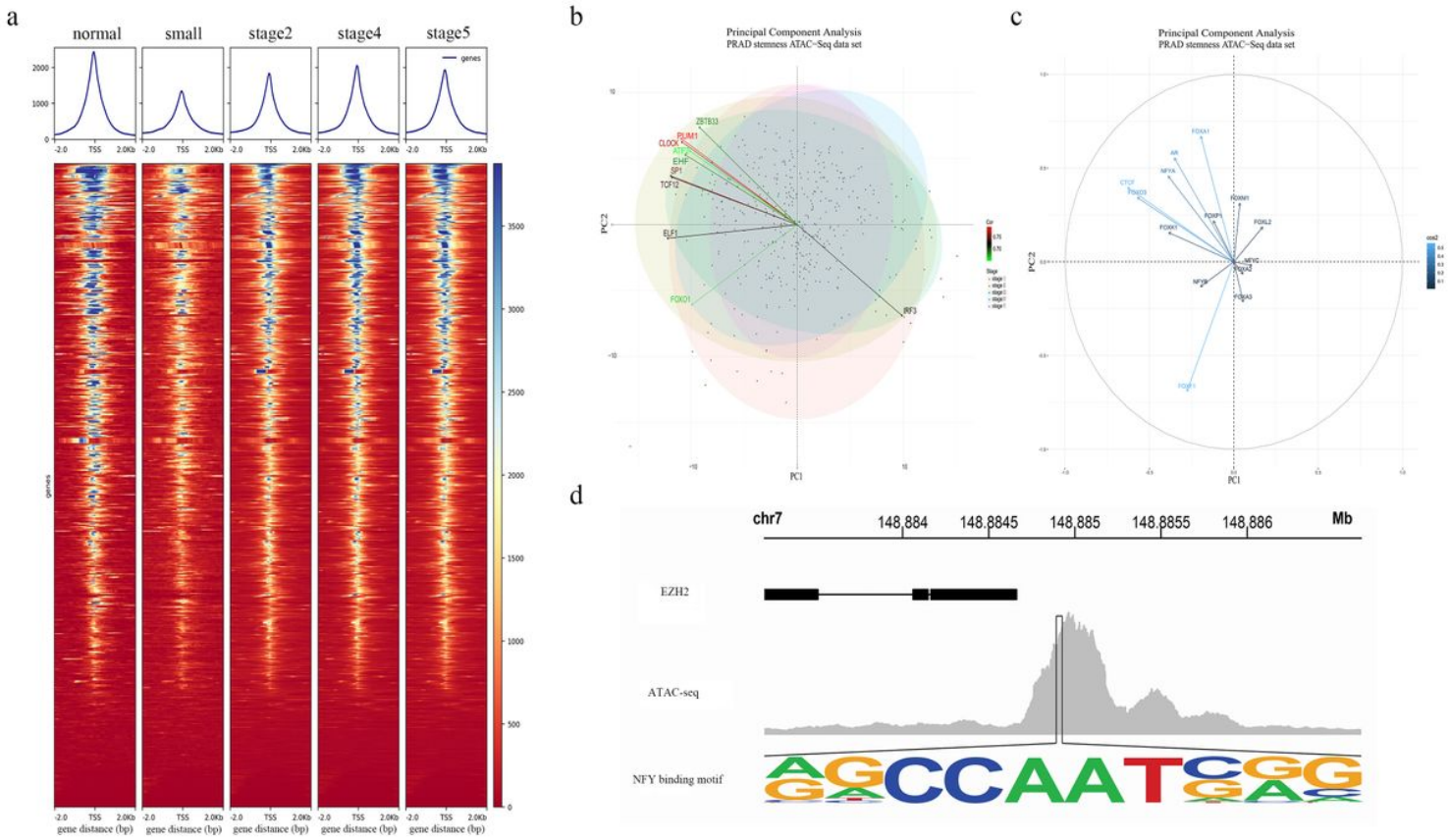

Figure 3

Transcriptional regulation of stemness gene in prostate cancer a In normal samples, cancer samples of different grades, and prostate small cell cancer samples, the transcription factor binding intensity of stemness gene. b The main transcriptional regulators are obtained based on PCA analysis. Red and green represent the strength and weakness of transcriptional regulatory factors, respectively. c Based on PCA analysis, the importance of known transcriptional regulators of stemness genes in prostate cancer. The shades of blue represent the correlation between transcription factors and stemness. D Motif map of transcription factor binding to EZH2.

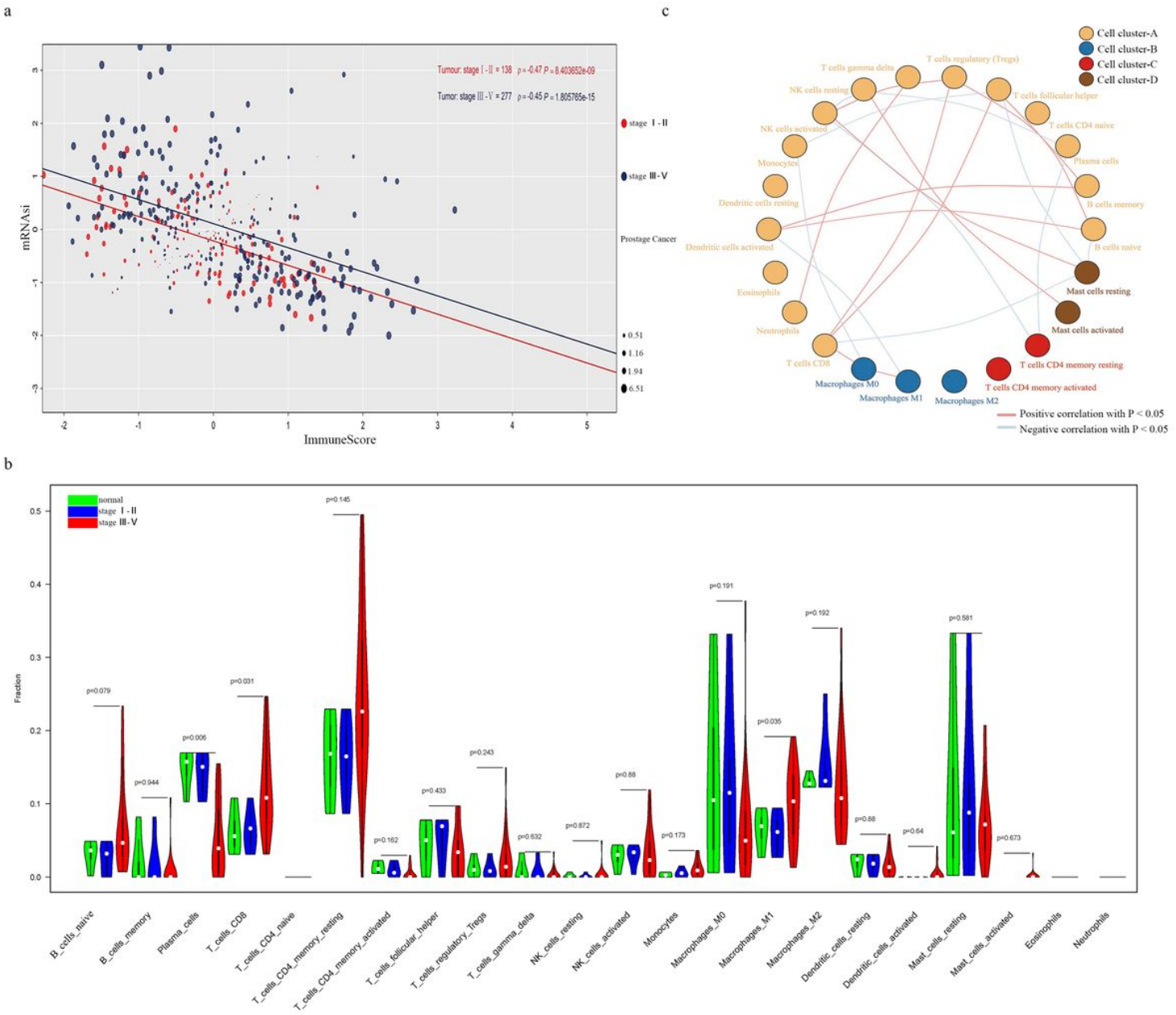

Figure 4

Stemness and immune cells in prostate cancer a In different clinical grades of prostate cancer, stemness score is negatively correlated with immune infiltration score. b In the prostate tumor microenvironment of different stages, the fraction of 22 types of immune cells. c Correlation network of 22 types of immune cells.

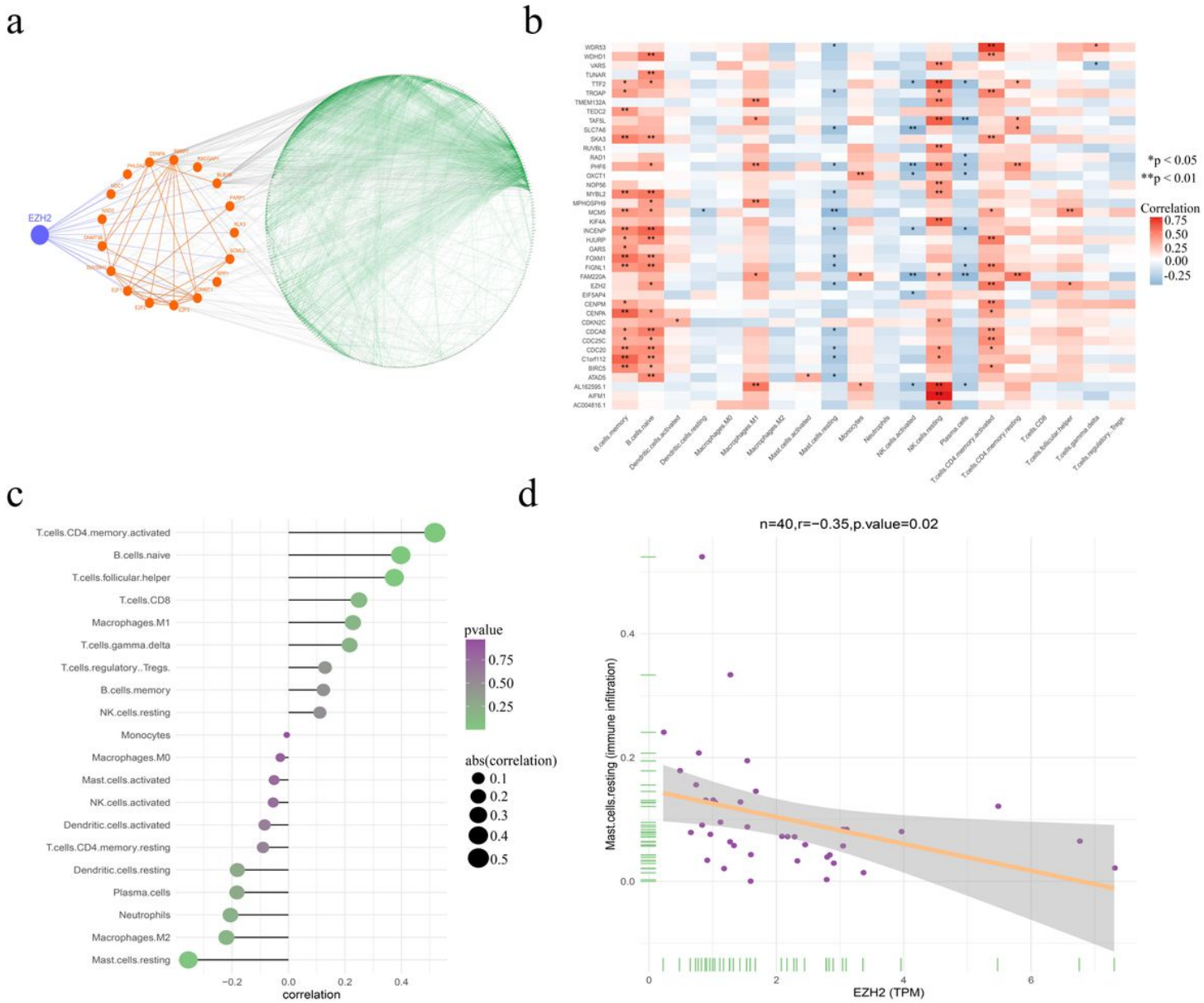

Figure 5

The protein interaction network of stemness gene, the relationship between stemness gene and immune infiltration a The connection between stemness genes and EZH2 in MEmagenta module. According to whether genes interact directly with EZH2, it is divided into a small circle and a large circle. The blue lines represent genes that directly interact with EZH2. The yellow lines represent the interaction between genes in the small circle, and the green lines represent the interaction between genes in the large circle. The gray lines represent the interaction of genes between the small circle and large circle. b The correlation between stemness gene and immune cells, red represents positive correlation, blue represents negative correlation c and d The correlation between EZH2 and immune cells, and the correlation between EZH2 and resting mast cell

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