Analysis and Validation of Key Genes Related to Radiosensitivity in Prostate Cancer

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Abstract

Purpose: To investigate the potential relationship between differential gene expression, biological function enrichment and disease prognosis affecting the sensitivity of prostate cancer radiotherapy by bioinformatics analysis.

Materials and Methods: Retrieve and obtain data on differential gene expression of prostate cancer radiosensitivity in the GEO database (GSM3954350, GSM3954351, GSM3954352), GER2 tool to screen and analyze the differential genes, Enrichr database for enrichment analysis of GO and KEGG, use Cytoscape software builds protein-protein interaction (PPI) networks and analyzes key genes.

Results: A total of 7043 differentially expressed genes were screenedout, including 3842 high expression genes and 3199 low expressed genes. The top 20 differentially expressed genes were selected for further analysis. Their biological functions are mainly enriched in the following aspects:“Cell communication” and “Signal transduction”; cytological components are mainlylocated outside the cell; molecular functions are enriched in structural molecular activity, receptor binding, serine-like peptidase activity, etc. The KEGG enrichmentanalysisshowedthat the differentially expressed genes were mainly enriched in the mismatch repair pathway, non-homologous terminal binding pathway and so on.Survival analysis showed that VGF gene was associated with the prognosis of prostate cancer patients receiving radiotherapy, and high expression of VGF significantly reduced progression-free survival(PFS) in these patients(HR=4.84, 95% CI: 1.34-17.5, P= .016).

Conclusion:This study identified key genes associated with radiation sensitivity in prostate cancer and

verified the relationship between the VGF gene and patient prognosis.

INTRODUCTION

Prostate cancer is a malignant tumor with a high incidence rate, currently highest in North America and Europe, followed by Asia and Africa. A study by Basiri et al. showed ethnic differences in prostate cancer risk in Iran, the most populous multi-ethnic country in the Middle East[1]. According to the American Cancer Society's epidemiological survey: the death rate of prostate cancer has been declining for more than 20 years, although this number has stabilized in recent years[2-3].The challenge for us is how to continue to reduce this number and enable prostate cancer patients to gain more benefit from treatment. Radiotherapy is playing an increasingly important role in the treatment of prostate cancer, with the continuous improvement of radiotherapy equipment and methods, its application in the treatment of various stages of prostate cancer has achieved good treatment result[4-5].Nevertheless, as tumor cells develop resistance to radiotherapy, its efficacy will gradually be limited, and the patient's tumor progression will follow[6-7]. The mechanism of radioresistance in prostate cancer cells still remains unclear, PI3K/Akt and mTOR signaling pathways, DNA repair, autophagy and epithelial mesenchymal transformation probably take part in this process[8]. Therefore, exploring the factors that affect the sensitivity of prostate cancer radiotherapy is of great significance to improve the therapeutic effect.

The Gene Expression Omnibus (GEO) of the National Center for Biotechnology Information is currently the largest and most comprehensive public gene expression database,it has been widely used in the study of differential gene expression in tumors[9]. In this study, we downloaded three microarray data sets from the GEO database to identify key genes that affect the sensitivity of prostate cancer radiotherapy and analyze their biological functions.

MATERIALSAND METHODS

Data acquisition

Search for publicly published gene chips in the GEO database (https://www.ncbi.nlm.nih.gov/geo/):①The search term is "prostate cancer", "radiotherapy"; ②The species is "human". Finally, three data sets wereobtained(GSM3954350,GSM3954351,GSM3954352).The experimental group was radiotherapy-insensitive patients, and the control group was radiotherapy-sensitive patients. Download the differentially expressed gene data and use the difference log fold change>2 and adjust<0.05 as the screening conditions for the difference genes.

Functional enrichment analysis

The differentially expressed genes were converted into names of the same type, and functional enrichment analysis was performed based on Geneontology (GO) and Kyoto Encyclopedia of Genesand Genomes (KEEG).Evaluate their functional associations through the String database (http://strin abstract db.org).The Degree Score greater than 0.4 is considered significant. The degree

for association of these genes was analyzed with Cytoscape software (version 3.6.0), and build a visual hub gene network was established

Statistical analysis

Statistical analysis was carried out with SPSS25.0 software, measurement data used T test; count data used chi-square test, P<0.05 indicated that there was a statistical difference. For Kaplan–Meier curves, p-values and hazard ratio (HR) with 95% confidence interval (CI) were generated by log-rank tests and univariate Cox proportional hazards regression. All analytical methods above and R packages were performed using R software version v4.0.3 (The R Foundation for Statistical Computing, 2020). $p < 0.05$ was considered as statistically significant.

RESULTS

Differential Gene Screening

We retrieved the GEO database for gene expression of prostate cancer patients with radioresistance after radiotherapy(GSM3954350，GSM3954351,GSM3954352),a total of 7043 differentially expressed genes were found, of which 3842 were highly expressed and 3199 were lowly expressed.Top 20(**Table.1**) genes with differential expression levels were selected for further analysis(**Figure.1**).

Among them, the genes with high expression were GCG, IL7R, GJA1, VGF, FGF13, GPR143, while t he genes with low expression wereIL7, CCL2, CSF3,IGFBP3, BDKRB1, ADCY5, GRPR, FLT3LG, ADRA2C, BMP7, GHR, LTBP1, EVA1A, QRFPR.

GO and KEGG enrichment analysis

GOenrichment analysis was used to analyze the biological process (BP) of the differential genes, suggesting that their functions may be mainly enriched in the following aspects (**Figure.2**):“Cell communication”（65%，P=0.004）and “Signal transduction”（65%，P=0.007）; Cytological components are mainly extracellular（24.8%, P=0.036） (**Figure.3**).Molecular functions(MF) are mainly enriched in structural molecular activity, receptor binding, serine-like peptidase activity, etc. (**Figure.4**).Enrichment analysis of KEGG signaling pathway showed that the differential genes mainly concentrated in the cytokine-cytokine receptor interaction, hematopoietic cell lineage and other pathways (**Figure.5**).

Protein-protein interactive analysis

Use the online analysis tool of the STRING database to evaluate the association with the screened genes[(10)],the correlation was further analyzed with Cytoscape (Version 3.6.0). A hub gene network was established (**Figure.6**), and the degree of association for hub genes and other genes were ranked (**Table.2**).These genes indicatepossible targets for radioresistance in prostate cancer.

Prognosis analysis

We obtained information on 41 prostate cancer patients who received radiotherapy and completed

RNA sequencing from the Cancer Genome Atlas (TCGA) database. We analyzed the expression levels of the above genes in these patient samples, and performed survival analysis using the Kaplan-Meier method.The results suggest that the expression of VGF gene is related to the prognosis of these patients. We drew the Kaplan-Meier curve of VGF gene (**Figure 7**). Thus, patients with high VGF expression had significantly lower progression-free survival (PFS) than those with low VGF expression.(HR=4.84, 95% CI: 1.34-17.5,P=.016)

Disscussion

With the in-depth research on prostate cancer, its treatment methods have become more efficient and diversified,the progress of treatment methods must be guided by the smallest risk in exchange for higher curative benefits.Protecting the body's functions as much as possible and improving the quality of life should be the objective of treatment.Due to recent research progress, for some prostate cancer patients with specific stages, radiotherapy has benefited more than 90% of them[11-13],however, for patients who relapse after receiving radiotherapy, the occurrence of radioresistance in cancer cells is a main reason[14]. The current prognosis and risk score system of prostate cancer still lacks the assessment of radiosensitivity,exploring the causes of radioresistance in prostate cancer cells will help improve the therapeutic effect and application range of radiotherapy[15].Radioresistance in prostate cancer is highly unpredictable and is currently thought to be driven by multiple cellular processes that allow cancer cells to adapt to routine radiation doses[16-17].In this study, we obtained 3 gene expression data sets on radiosensitivity of prostate cancer from GEO database, and screened out the top 20 differentially expressed genes, among which 6 genes were up-expression (GCG, IL7R, GJA1, VGF, FGF13, GPR143) and 14 genes were decreased expression(IL7, CCL2, CSF3,IGFBP3, BDKRB1, ADCY5, GRPR, FLT3LG, ADRA2C, BMP7, GHR, LTBP1, EVA1A, QRFPR).The biological processes of these differential genes are mainly enriched in the following aspects: “Cell communication” and “Signal transduction”.The enrichment analysis of KEGG signaling pathway showed that these differential genes were mainly concentrated in cytokine-cytokine receptor interaction, hematopoietic cell lineage and other pathways, and the results of GO analysis suggest that gene products are enriched in the extracellular matrix during the performance of their functions. This provides a general direction for our next research: for example, during cell-cell communication and signal transduction, some different genes activate some unusual functions that lead to radioresistance of tumor cells.Raw counts and corresponding clinical information were obtained from The Cancer Genome Atlas (TCGA) dataset (https://portal.gdc.com) of RNA sequencing data (level 3) from 41 patients with prostate cancer who underwent radiotherapy. Log rank was used to test KM survival analysis to compare survival differences between the two or more groups mentioned above. high expression of VGF gene was found to significantly shorten PFS in prostate cancer patients receiving radiotherapy,however, the specific mechanism of action still needs further experimental verification. The study by Seifertetal[15].showed that prostate cancer patients with high expression of VGF and low

expression of GRPR had a higher recurrence rate,they also demonstrated in vitro that inhibition of VGF expression can improve the sensitivity of radiotherapy, which was consistent with our research results. In fact, VGF was closely related to the prognosis of various cancers[18-19], therefore, we speculate that the correlation between VGF and radiosensitivity may be related to the epithelial-mesenchymal transition (EMT) induced by it[20]. In addition, although previous studies have confirmed that CCL2 is a key factor involved in the invasion and metastasis of prostate cancer [21]. However, mai et al. found that the high expression of CCL2 was not only significantly correlated with the recurrence of nasopharyngeal carcinoma(NPC), but also that inhibition of CCL2 could enhance the radiosensitivity of NPC cells. Combined with the study of Kalbasi et al[22]. in pancreatic cancer, it could be speculated that:CCL2 may promote the radioresistance of cancer cells by recruiting monocytes/macrophages, and the specific mechanism of which deserves further analysis.These researchers have made great contributions to the research in this field, and other hub genes obtained in this study are also valuable for further research.we noticed that among the hub genes obtained in this study, there are many genes related to blood glucose regulation and human immune system regulation, such as GCG, ADCY5, IGFBP3, CSF3, CCL2, IL7R, etc, which also provides potential directions for further research. The next step, however, must demonstrate a causal relationship between radiosensitivity and tumor progression: that is, radiosensitivity leads to tumor progression rather than poor radiation response due to tumor progression. Currently, there is a lack of studies on the use of radiotherapy alone for prostate cancer, so there are many biases in the study of the effects of radiotherapy, which also need to be addressed.

CONCLUSION

Through the analysis of the differential genes related to the sensitivity of prostate cancer, the key genes found in this study can help to understand the potential molecular mechanism of prostate cancer radioresistance.By combining clinical data from TCGA database, we confirmed that high expression of VGF gene can significantly reduce PFS in prostate cancer patients who underwent radiotherapy.

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Table.120 differentially expressed genes

Gene	LogFC	P.valve
GCG	5.50042646	3.07E-07
IL7	-4.55450931	1.86E-06
CCL2	-4.31805385	6.30E-06
CSF3	-3.75601818	7.42E-06
IL7R	5.01631541	4.28E-05
IGFBP3	-3.69658348	8.43E-05
BDKRB1	-3.69396659	8.79E-05
GJA1	4.82279894	9.96E-05
ADCY5	-3.47914908	1.15E-04
GRPR	-3.23563766	1.27E-04
FLT3LG	-3.20223477	1.54E-04
ADRA2C	-3.17089892	1.63E-04
BMP7	-3.10999178	2.03E-04
VGF	3.63181712	2.53E-04
GHR	-3.06502295	2.92E-04
FGF13	2.93364754	3.16E-04
LTBP1	-3.03787139	3.25E-04
EVA1A	-2.95634334	3.37E-04
QRFPR	-2.88320079	3.42E-04
GPR143	2.79000942	3.56E-04

Table.2 Hub gene sequencing

Rank	Name	Score
1	GCG	13
2	IL7	10
2	CCL2	10
4	CSF3	9
5	IL7R	8
6	IGFBP3	7
6	BDKRB1	7
6	GJA1	7
9	ADCY5	6
9	GRPR	6

9	FLT3LG	6
12	ADRA2C	5
12	BMP7	5
12	VGF	5
12	GHR	5
12	FGF13	5
17	LTBP1	4
17	EVA1A	4
17	QRFPR	4
17	GPR143	4

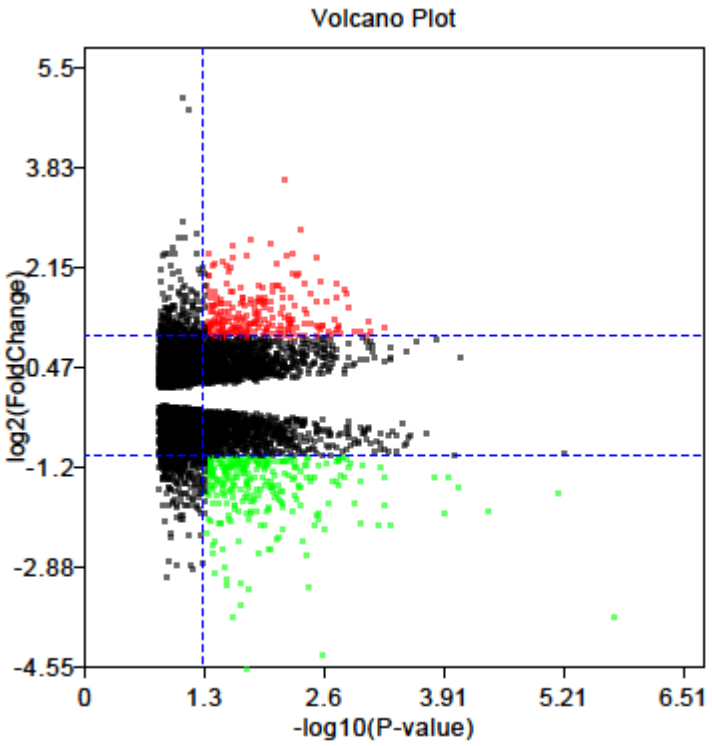

Figure.1 Differential gene expression volcano map of GSM3954350, GSM3954351, GSM3954352 data sets（Thered dots represent genes with significantly up-regulated expression, green dots represent genes with significantly down-regulated expression, and black dots represent genes with no differentially expressed expression）

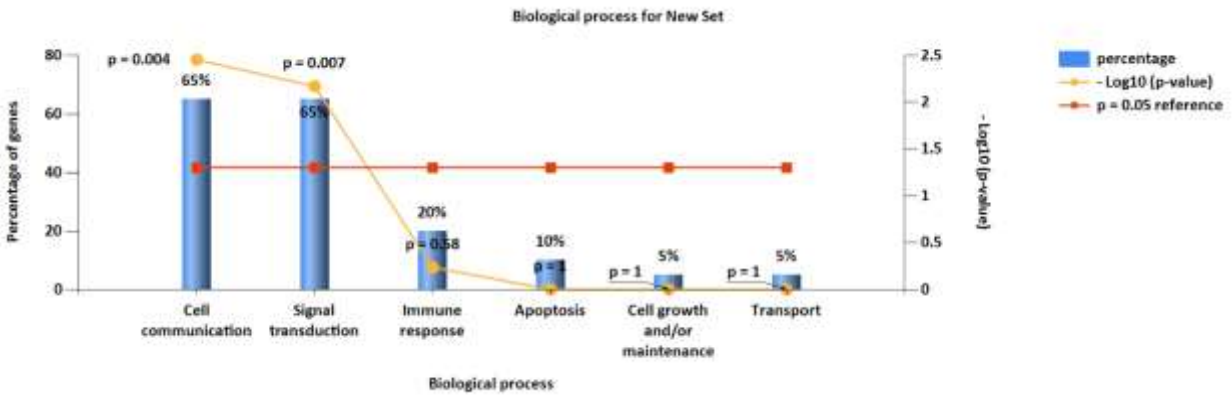

Figure.2 GO Analysis of Differential Gene: Biological Process（Biological processes(BP) are significantly enrichedin“Cellcommunication”and“Signal transduction”）

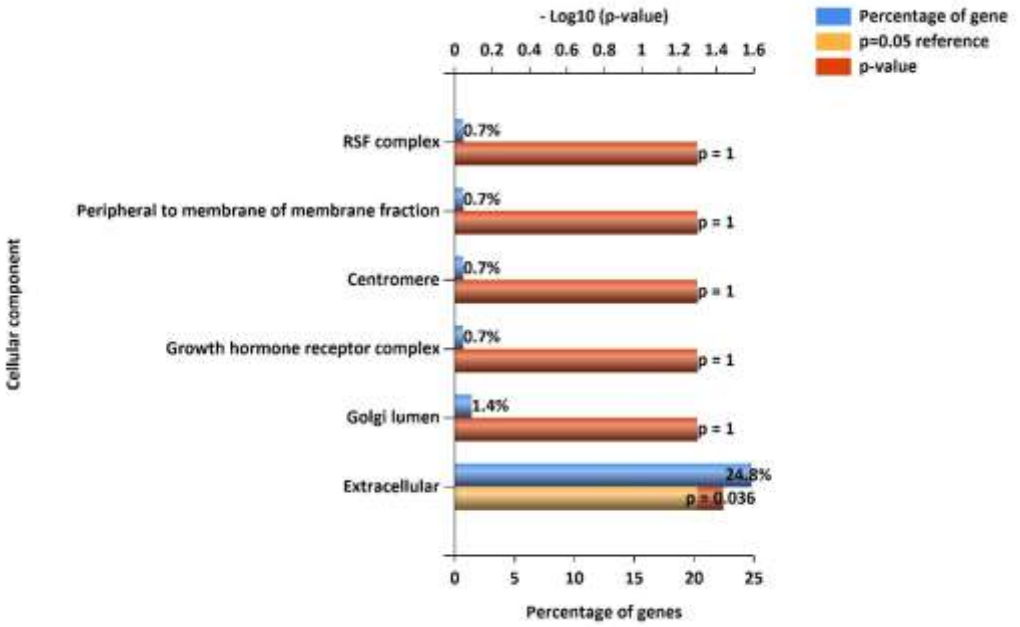

Figure.3 GO Analysis of Differential Gene: Cellular Components（cellular component (CC) annotation showed that the gene products mainly enriched in the extracellular,24.8%,P=0.036）

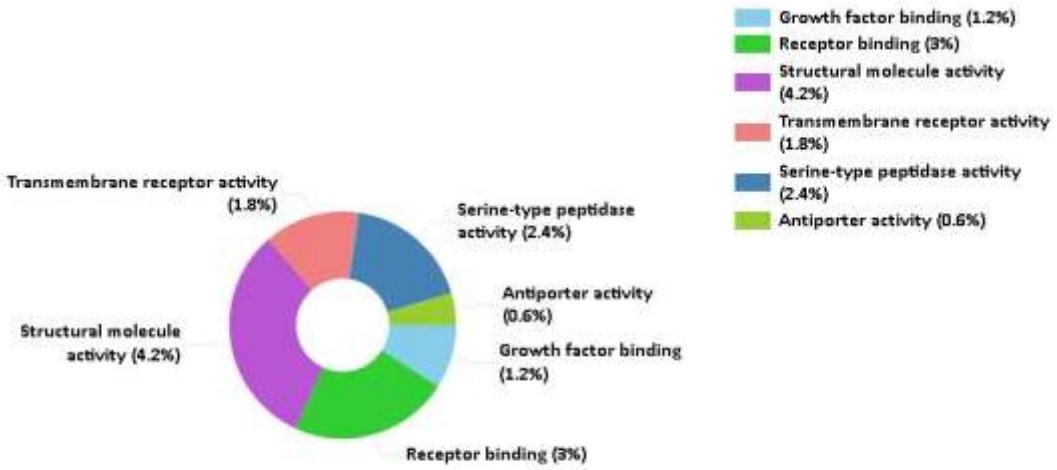

Figure.4 GO Analysis of Differential Gene: Molecular Function(molecular function (MF) annotation showed that “structural molecular activity”, “receptor binding”, “serine-like peptidase activity” were the most enriched term with 6 gene hits,respectively**)**

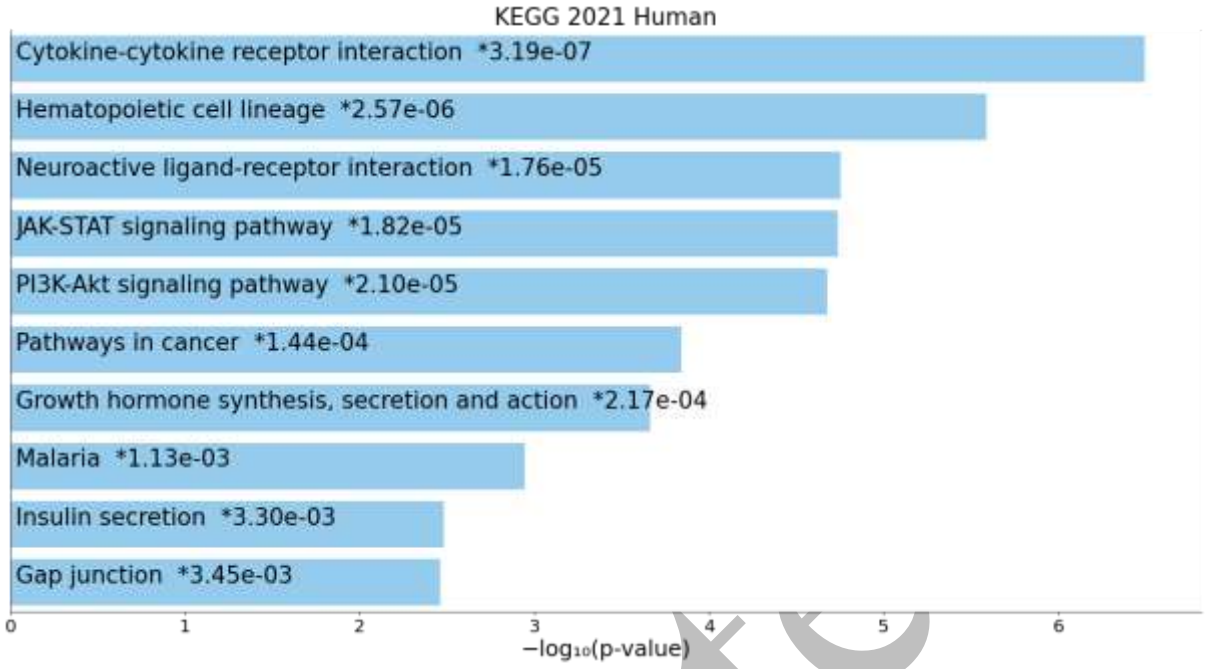

Figure.5 Enrichment analysis of KEGG signaling pathway of differential genes（KEGG pathway enrichment analysis: the top 10 pathways with significant enrichment of differential genes, *stands for p-value）

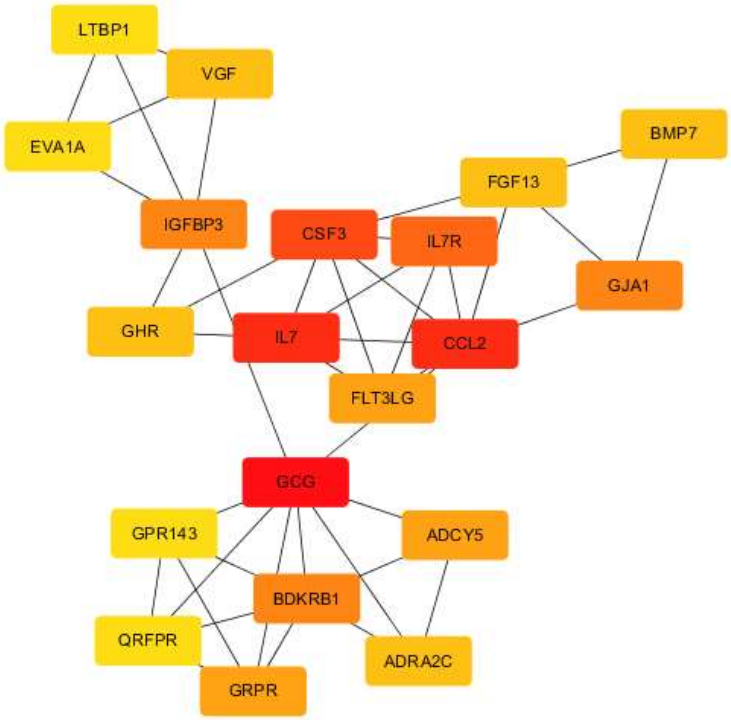

Figure.6 Hub Gene Network

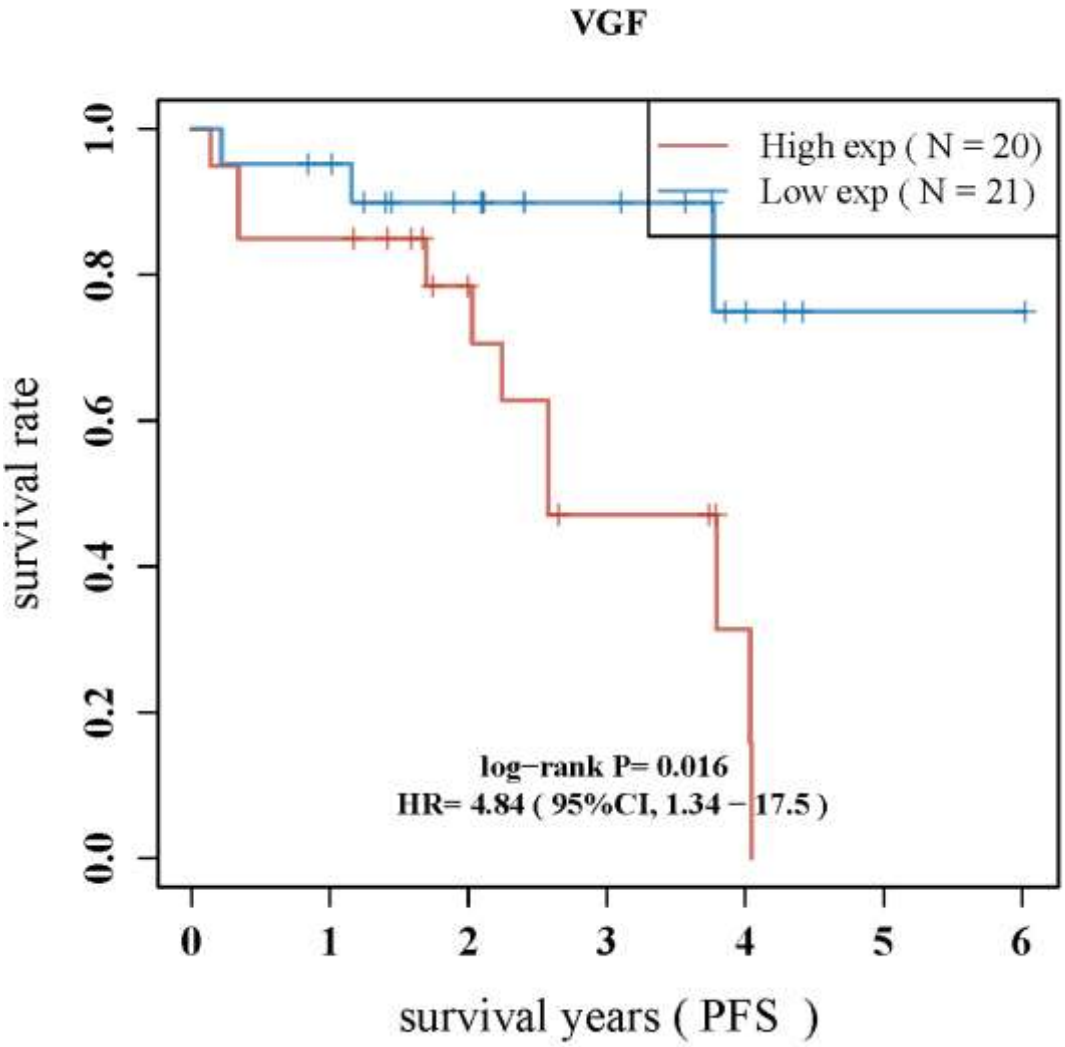

Figure.7 Kaplan-meier survival analysis of VGF gene