The E545 Mutation of PIK3CA in Cervical Cancer Promotes the Survival by Several Different Pathways

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Research Article

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Abstract

Backgroud: The E545 mutation of PIK3CA in Cervical cancer is frequently happened. But the role of E545 mutation of PIK3CA in Cervical cancer is not clear.

Methods: In this study, we analysised the molecular signatures of E545 mutation Cervical cancer by bioinformatics methods.

Results: We collected transcriptome sequencing results of 227 no mutation cervical cancer tissue samples and 36 mutation cervical cancer tissue samples, then analyzed the data combining bioinformatics methods. A total of 5 differential expression miRNAs were obtained, including 3 up-regulated miRNAs, 1 down-rugulated miRNA. A total of 174 differential expression genes were obtained, including 132 up-regulated genes, 40 down-rugulated genes. GO analysis suggested that the up-regulated DEGs were mainly enriched in transcription factor activity, leukotriene signaling pathway and so on. Besides, we constructed a PPI network with DEGs to screen the top hub genes with a relatively high degree of connectivity. Among them CAV1, KRT20, FOS, had a degree of connectivity larger than 5 and functioned as hub module genes to promote the survival of E545 mutation cervical cancer. We also identified different miRNA-DEG axis, including hsa-mir-449a-AXL, hsa-mir-508-CGA, COL15A1, NNMT, hsa-mir-552-CHST6, NWD1. These axis regulated the survival of E545 mutation cervical cancer togetherly.

Conclusions: In conclusion, this study identified DEGs and screened the key genes and pathways closely related to E545 mutation in Cervical cancer by bioinformatics analysis, These results might hold promise for finding potential therapeutic targets of cervical cancer harboring E545 mutation of PI3KCA.

Background

Cervical cancer is the fourth most common female malignancy worldwide, human papilloma virus (HPV) infection is the main cause of the disease in most cases. Approximately 90% of cervical cancers occur in poor countries and regions because of lacking screening and prevention[1], until now, the treatment of Cervical cancer is surgery or radiation therapy, supplemented by chemotherapy. Increasing studies have focused on exploring the molecular characteristics of cervical cancer to identify new therapeutic targets to improve the prognosis of this disease.

The PIK3CA gene, coding for the catalytic subunit p110alpha of classIA phosphatidylinositol 3-kinases (PI3Ks), is essential for various biological processes, including cell proliferation, invasion and migration, differentiation, apoptosis, and glucose metabolism[2,3]. PIK3CA mutation has been observed in various solid tumors[4], In cervical cancer, PIK3CA has been identified as one of the most commonly mutated genes, and the mutation rate ranges from 10 to 30%, three prevalent mutants of PIK3CA are E542K, E545K, and H1047R, compared to other two mutations, E545K mutation occurred in higher frequency, most studies considered a mutation of PIK3CA promoted cancer progression based on cell experiment, lacking animal model or clinical data. Because of the complexity of the PIK3 pathway, so its role in Cervical cancer is elusive, it is necessary to ascertain the role of PIK3CA mutation in Cervical cancer.

MicroRNAs (miRNAs) are short non-coding RNAs (ncRNAs) of ~ 22 nucleotides that mediate gene silencing by guiding Argonaute (AGO) proteins to target sites in the 3' untranslated region (UTR) of mRNAs[5], resulting in miRNA-induced silencing complex (miRISC), which promotes translation repression and degradation of targeted mRNAs[6]. miRNAs are involved in Cervical cancer progression by targeting different pathways, for example, MIR-G-1 mediated the activation of canonical WNT-CTNNB1/β-catenin signaling pathway, and accelerated malignant behavior[7], miR-146b-3p promotes cervical cancer cell proliferation, migration, and anchorage-independent growth through activation of STAT3 and AKT pathways[8].

In this study, We collected transcriptome sequencing results of 227 no mutation cervical cancer tissue samples and 36 mutation cervical cancer tissue samples and analyzed the data by bioinformatics methods, we found the E545 mutation of PIK3CA promoted the survival of cervical cancer by different axis, including miRNA-target axis (miR449a-AXL, miR508-CGA, COL15A1, NNMT, miR552-CHST6, NWD1), Up-regulated genes, down-regulated genes, they acted together in promoting the survival of Cervical cancer patients.

Materials And Methods

TCGA data

The gene expression profiles of the TCGA dataset were downloaded from the TCGA database. The TCGA dataset contained 263 samples, including 36 cervical cancer samples(mutation of PI3KCA) and 227 cervical cancer samples(no E545 mutation of PI3KCA).

Screening for differentially expressed genes and miRNAs

The statistical software R and packages from Bioconductor (http://www.bioconductor.org/biocLite.R) were used to analyze the differentially expressed genes and miRNAs between the mutation samples and no mutation samples. Before analysis of the data, Use software R to combine data according to the type of sample, the microarray data were quality tested. In this process, R packets, including packages of GDCRNAtools, packages of NA, packages of edgeR, packages of limma were used. According to the Limma (http://www.bioconductor.org/packages/release/bioc/html/limma.html) package of Bioconductor, key differentially expressed genes and miRNAs were determined. $P < 0.05$ and $|\log FDR| \geq 1$ was considered to indicate a statistically significant difference.

Functional and pathway enrichment analysis

Using the online database David (https://david.ncifcrf.gov/) to carry out functional and pathway analysis, import the differentially expressed genes into the database, then plot in software R by using packages of ggplot2. GO terms are divided into three categories, including Biological process, cellular component, and molecular function.

Screening of miRNA targets

Predict the target gene by the online database targetscan, miRDB. Then these genes Intersect with differentially expressed genes and relatively reliable target genes can be obtained.

Survival analysis

Kaplan-Meier Plotter (http://kmplot.com/analysis/) contains survival data for 7489 patients from 21 cancer studies from TCGA. The tool was used to conduct an overall survival analysis (OS) for patients with cervical cancer, miRNA and genes can be analyzed, some survival analysis by GEPIA(http://gepia.cancer-pku.cn/), Log-rank P-values in survival analysis were recorded, 50th (upper) percentiles and 50th (lower) percentiles were considered as high and low groups.

PPI network

The STRING database (http://www.bork.emblheidelberg.de/STRING/) is a computerized and powerful global resource for studying the interactions between the experimental and predicted interactions of proteins. In the present study, the DEGs expression genes were constructed using the STRING database to construct PPI networks, and a combined score of ≥ 0.4 was used as the cut-off value. A complex network of DEGs was constructed, the excel of string-interactions was downloaded and saved in a CSV file. String-interactions file and DEGs expression file were imported into Cytoscape software for further processing.

Results

The E545 mutation of PI3KCA promoted the survival of Cervical cancer patients

In order to confirm the role of E545 mutation in Cervical cancer, we first used the online database ICGC (https://dcc.icgc.org/) to detect if E545 mutation affected the survival of Cervical cancer patients, the ICGC database had information of 38 mutation patients and 256 no mutation patients, the overall survival rate was higher in mutation patients(data not shown). However, because of the limited numbers of mutation cancer patients, the P-value was not significant. Libing Xiang reported the mutation of PI3KCA promoted the survival of cervical cancer patients[9]. His data included 105 mutation samples, 666 no mutation samples (Fig. 1). so we next wanted to dissect the mechanism of mutation of PI3KCA promoting the survival of cervical cancer.

Screening for differentially expressed genes and miRNAs

We collected transcriptome sequencing data of 36 mutation cervical cancer tissue samples and 227 no mutation cervical cancer tissue samples from TCGA database, then combined data according to the type of sample by software R and analysis by bioinformatics methods. A total of 174 differential expression genes were obtained in mutation Cervical cancer samples compared with no mutation Cervical cancer samples, including 132 up-regulated genes, 40 down-regulated genes, as displayed in the volcano plot (Fig. 2B), the top 20 upregulated genes and downregulated genes were shown in Table 2, 3, including

gene names, expression fold changes, and P values. A total of 4 differential expression miRNAs were obtained, including 3 up-regulated miRNAs, 1 down-regulated miRNA, as displayed in the volcano plot (Fig. 2A) and Table1.

Table 1
Detailed information on the differentially expressed miRNAs in the analysis result, including gene name, expression fold change, and statistics, such as P value .

Differentially expressed miRNA	logFC	PValue
hsa-mir-202	2.265131	8.26E-14
hsa-mir-449a	2.8466801	1.97E-12
hsa-mir-508	1.9153177	1.35E-05
hsa-mir-449b	1.9289771	4.82E-05
hsa-mir-552	-3.6661082	0.0001215

Table 2
Detailed information on the up-regulated expressed genes in the analysis result, including gene name, expression fold change, and statistics, such as P value.

up-regulated gene name	logFC	PValue
KRT79	4.395124825	5.62E-30
PSKH2	3.801897881	3.14E-19
TDRG1	3.160472574	1.09E-15
CXorf67	3.061968642	1.59E-10
CAMP	2.913997686	2.60E-19
C20orf85	2.61386283	8.25E-07
SNAI3	2.597794072	5.50E-27
DPEP3	2.49391072	8.01E-09
COX4I2	2.356928335	1.93E-20
MATN3	2.339405862	3.38E-16
SLC18A3	2.327800026	4.66E-05
FAM181A	2.326576057	3.46E-09
C1orf189	2.129572972	1.62E-11
DLK1	2.107279335	3.49E-07
RNU4-1	2.090945352	9.09E-11
CHAT	2.049378282	3.66E-08
ERVH48-1	2.040669862	9.09E-11
CAPN6	2.017512722	3.66E-08
AGMO	1.970675447	6.95E-08
LRRC17	1.954537237	4.13E-11

Table 3
Detailed information on the down-regulated expressed genes in the analysis result, including gene name, expression fold change, and statistics, such as P value.

down-regulated gene name	logFC	PValue
COL15A1	-7.902584	3.16E-05
REG1A	-6.31045198	3.18E-05
PNMT	-6.00908181	5.11E-07
CGA	-5.3949446	2.33E-05
MUC6	-5.18472717	1.66E-05
PLA2G2A	-4.64021733	4.29E-07
KRT20	-4.59001751	4.36E-05
TFF2	-3.98849464	7.72E-05
CLDN6	-3.91211409	2.19E-05
PAEP	-3.82223355	0.000116824
ALDH8A1	-3.7874588	1.07E-05
SPRR2G	-3.55228403	7.35E-05
SFTA1P	-3.42605159	1.07E-05
KLK14	-3.41141444	1.14E-07
CPS1	-3.359905	0.000149894
DIO3	-3.32019664	8.25E-08
LGALS4	-3.11579128	6.03E-05
FOLR3	-2.83018414	0.000130534
TESC	-2.78487362	2.83E-06
CDH17	-2.78372222	0.000110485

Functional and pathway enrichment analysis

GO term enrichment analysis found an obvious quantity variance and significance level difference among the 174 DEGs that were enriched in biological processes, molecular functions, and cellular components. For cellular components, the DEGs were significantly enriched in the extracellular region, insoluble fraction, membrane fraction, for molecular function, the DEGs were significantly enriched in leukotriene receptor activity, structural molecular activity (Fig. 4B). As for GO pathway, the up-regulated DEGs were mainly enriched in transcription factor activity, leukotriene signaling pathway, positive

regulation of transcription from RNA polymerase II promoter, response to corticosterone, and G-protein coupled peptide receptor activity, these DEGs could act as stress action playing a negative role in the malignant proliferation of Human cervical carcinoma (Fig. 3A). The down-regulated DEGs were mainly enriched in extracellular space, negative regulation of BMP signaling pathway, structural molecule activity, negative regulation of plasma membrane long-chain fatty acid transport, and response to hypoxia, these pathways may play a negative role in the proliferation of cervical cancer (Fig. 3B).

Construction of PPI network

PPI information was acquired from the STRING database. A PPI network graph was conducted by Cytoscape software, Specifically, the network contained 74 nodes (genes) and 72 edges (interaction), the red circle represented up-regulated genes, the yellow circle represented down-regulated genes. Among them CAV1, KRT20, FOS, had a degree of connectivity larger than 5 and could function as hub module genes (Fig. 4A), Caveolin-1 functions as a membrane adaptor to link the integrin alpha subunit to the tyrosine kinase Fyn, it is necessary to couple integrins to the Ras-ERK pathway and promote cell cycle progression[10], besides, Caveolin-1 also plays an important role in tumor cell invasion[11], CK20 detection in urine cells is a simple, noninvasive method with a high potential to become the marker of choice for monitoring and follow-up of TCC patients, the high expression of KRT20 had a poor Prognosis[12], hepatocyte-specific deletion of c-Fos protects against diethylnitrosamine (DEN)-induced HCCs, whereas liver-specific c-Fos expression leads to reversible premalignant hepatocyte[13], Taken together, these nodes functioned together in the progress of cervical cancer.

miRNA-DEG axis

The target genes of the validated differential expression miRNAs in mutation cervical cancer were performed by online databases targetscan or miRDB. Given miRNAs negatively regulating the expression of their targets, we analyzed the correlation of upregulated genes with downregulated miRNAs, downregulated genes and upregulated miRNAs, Then we Intersected miRNA respectively with up-regulated or down-regulated genes, AXL was the target gene of hsa-mir-449a, CGA, COL15A1, NNMT were the target genes of hsa-mir-508, CHST6, NWD1 were the target genes of hsa-mir-552. Then, we made the visual networks of hsa-miR-449a(Fig. 5A), hsa-miR-508 (Fig. 5B), hsa-mir-552(Fig. 5C) and their targets by powerpoint software.

Survival analysis

In this study, the overall survival of patients with cervical cancer was analyzed by kmplot. We found the high expression of hsa-miR-449a was associated with high overall survival rate(log-rank P = 0.12) and the high expression of AXL was associated with poor overall survival rate (Fig. 6A, 6B). The high expression of hsa-miR-508 was associated with a high overall survival rate, the three targets of hsa-miR-508, the high expression of CGA, NNMT, COL15A1 was associated with poor overall survival rate (Fig. 6C-6F). The high expression of hsa-miR-552 was associated with poor overall survival rate, the two targets of hsa-miR-552,

the high expression of CHST6 and NWD1 was associated with a high overall survival rate(Fig. 6G-6I). Besides, the down-regulated genes had a high survival rate (Table 3, MUC6, TFF2, CAV1, KRT20, Fig. 7C-F), the up-regulated genes also had a high survival rate (Table2, KRT79, TDRG1, Fig. 7A-B).

Discussion

Cervical cancer is acknowledged as the most prevalent gynecological tumor and accounts for a large proportion of cancer-associated mortalities, in cervical cancer, PIK3CA has been identified as one of the most commonly mutated genes. It codes for p110α, the catalytic subunit of the phosphoinositide 3-kinase alpha (PI3Kα) complex, which is necessary for normal growth and proliferation[14]. Jiang W reported E545K mutation of PI3KCA confers radioresistance to cervical cancer cells[15]and promotes glycolysis and proliferation via induction of the β-catenin/SIRT3 signaling pathway in cervical cancer[16]. However, Xiang L reported The 3-year relapse-free survival was 90.2% for PIK3CA mutant patients and 80.9% for PIK3CA wild-type patients (P = 0.03). PIK3CA mutation was confirmed as an independent predictor for better treatment outcomes in the multivariate analyses (HR = 0.54, 95% CI: 0.29–0.99, P = 0.048)[9].

In this study, we found the E545 mutation of PIK3CA promoted the survival of cervical cancer. the up-regulated DEGs were mainly enriched in transcription factor activity, leukotriene signaling pathway, positive regulation of transcription from RNA polymerase II promoter, response to corticosterone, and G-protein coupled peptide receptor activity, Inoue M [17]reported the up-regulated transcription factor ATF3 inhibited the migration or invasion of HCT116 cells, it acts as a negative regulator of the migration and invasion of HCT116 human colon cancer cells exhibiting aberrant Wnt/β-catenin activity. Park JA studied that FosB Functions as Pro-Apoptotic Protein in Piperlongumine Treated MCF7 Breast Cancer Cells[18]. Downregulation of CAMK2B by promoter hypermethylation in breast cancer cells, implying CAMK2B may be a tumor suppressor[19], the upregulation of CAMK2B in mutation Cervical cancer suggested that E545K mutation of PI3KCA may cause Epigenetic changes. Reduced expression levels of KLF12 results in increased ability of lung cancer cells to form tumors in vivo and is associated with poorer survival in lung cancer patients, KLF12 can serve as a novel metastasis-suppressor gene[20]. The down-regulated DEGs were mainly enriched in extracellular space, negative regulation of BMP signaling pathway, structural molecule activity, negative regulation of plasma membrane long-chain fatty acid transport, and response to hypoxia. The main downregulated gene VEGFC promotes cervical cancer metastasis via up-regulation and activation of RhoA/ROCK-2/moesin cascade[21]. DKK1 promotes migration and invasion of hepatocellular carcinoma[22] and non-small cell lung cancer [23]. IGFBP6 gene plays an important role in the pathogenesis of breast cancer, Knockdown of IGFBP6 gene reduced migration activity of MDA-MB-231 cells[24]. In brief, the enriched pathways explained the E545K mutation of PI3KCA promoted the survival of cervical cancer patients.

We then constructed a PPI network with DEGs to screen the top hub genes with a relatively high degree of connectivity. Among them, CAV1, KRT20, FOS, had a degree of connectivity larger than 5 and could

function as hub module genes. Caveolin-1 functions as a membrane adaptor to link the integrin alpha subunit to the tyrosine kinase Fyn, it is necessary to couple integrins to the Ras-ERK pathway and promote cell cycle progression[10]. In our study, the downregulation of Caveolin-1 suggested it may suppress the cell cycle progression of cervical cancer cells. The high expression of KRT20 had a poor Prognosis[12], implying KRT20 may facilitate the progress of cervical cancer, the downregulation of KRT20 may inhibit tumor development. The miRNA-DEG axis, including hsa-mir-449a-AXL, hsa-mir-508-CGA, COL15A1, NNMT, hsa-mir-552-CHST6, NWD1. Buurman R reported[25] HCC cells transfection with miR-449 reduced expression of c-MET and phosphorylation of extracellular signal-regulated kinases 1 and 2 (downstream effectors of c-MET), increased apoptosis, and reduced proliferation and expression of miR-449 slows growth of HCC xenograft tumors in mice. AXL has been intrinsically linked to epithelial-mesenchymal transition (EMT) and promoting cell survival, anoikis resistance, invasion, and metastasis in several cancers[26]. hsa-mir-449a-AXL axis may play a role in curbing the tumor progression. MicroRNA-508 suppresses epithelial-mesenchymal transition, migration, and invasion of ovarian cancer cells through the MAPK1/ERK signaling pathway[27]. COL15A1 mRNA was up-regulated in HCC[28] and increased expression of several collagen genes is associated with drug resistance in ovarian cancer cell lines[29]. NNMT enhances the capacity of tumorigenesis associated with the inhibition of cell apoptosis and the promotion of cell cycle progression in human colorectal cancer cells[30] and serves as a potential biomarker for worse prognosis in gastric carcinoma[31]. These results suggested the microRNA-508 axis plays a negative role in the expansion of cervical cancer. MicroRNA-552 promotes hepatocellular carcinoma progression by downregulating WIF1[32]. Ectopic expression of miR552 enhanced cell proliferation, colony formation and resistance to drug-induced apoptosis in colorectal cancer[33]. CHST6 was found to be significantly related to the overall survival of cervical cancer[34]. Previous research has found that Nwd1 was expressed in neural stem/progenitor cells and was most analogous to the apoptosis regulator Apaf1, which may be involved with signaling molecules of axonal outgrowth regulation and promoting axon growth or apoptosis[35], implying it may promote the apoptosis of cervical cancer cells. However, the function of Nwd1 in cervical cancer was not clear.

In conclusion, our results identified several signaling pathways and several miRNA-DEGs axes, which may account for the survival of cervical cancer patients of E545 mutation of PI3KCA. Targeting these targets may hold potential for the treatment of cervical cancer of E545 mutation of PI3KCA.However, there are some limitations in our study, we need to verify the results in clinical samples obtained from patients.

Conclusions

In conclusion, our findings suggested that the E545 mutation of PIK3CA in Cervical cancer promotes the survival by several different pathways.

Declarations

Acknowledgements

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Authors' contributions

YC analysed the data and wrote the original manuscript. MY, JS, XZ, NH, QS, FQ helped collected data, JW carefully revised and discussed the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

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

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Figures

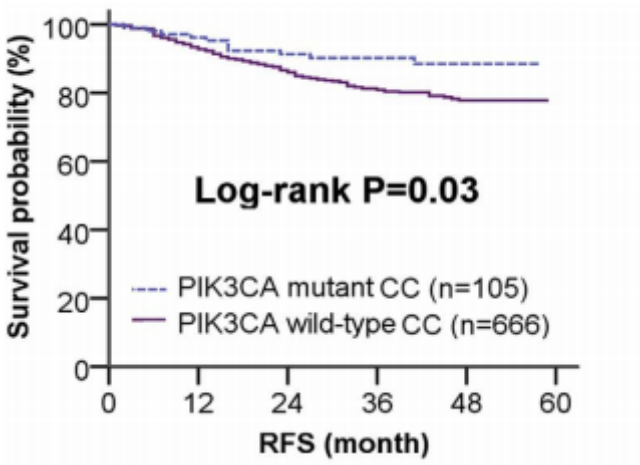

Figure 1

The survival curve of Cervical cancer harboring the E545 mutation of PIK3CA cited the data published by Libing Xiang9.

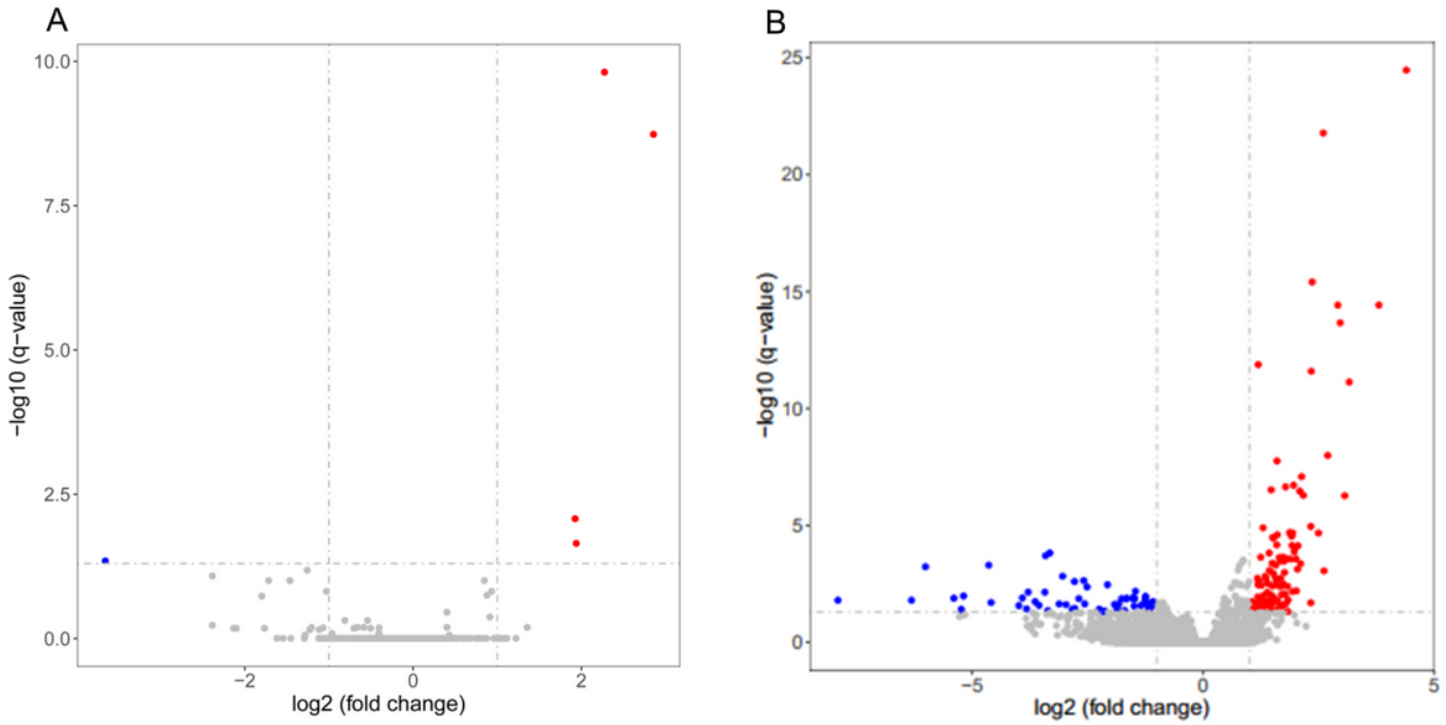

Figure 2

volcano plot (A) Volcano plot of differentially expressed miRNA, red represented up-regulated miRNAs, blue represented down-regulated miRNAs. (B) Volcano plot of differentially expressed genes, red represented up-regulated genes, blue represented down-regulated genes.

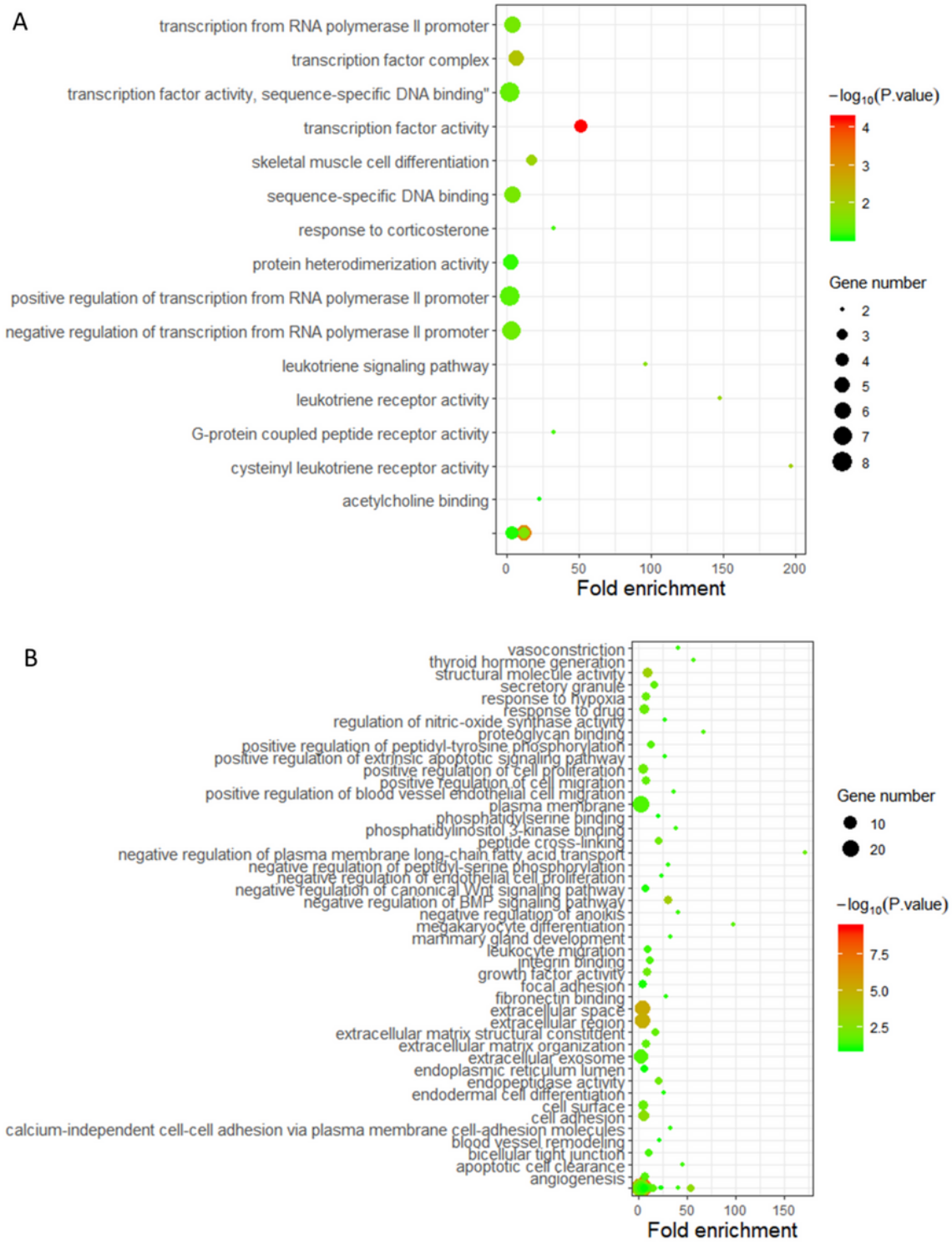

Figure 3

GO terms enrichment analysis of DEGs.(A) GO terms enrichment analysis of up-regulated genes, which were significantly enriched in transcription factor activity, leukotriene signaling pathway, positive regulation of transcription from RNA polymerase II promoter, response to corticosterone. (B) GO analysis results of down-regulated DEGs, Which were significantly enriched in extracellular space, negative regulation of BMP signaling pathway, response to hypoxia.

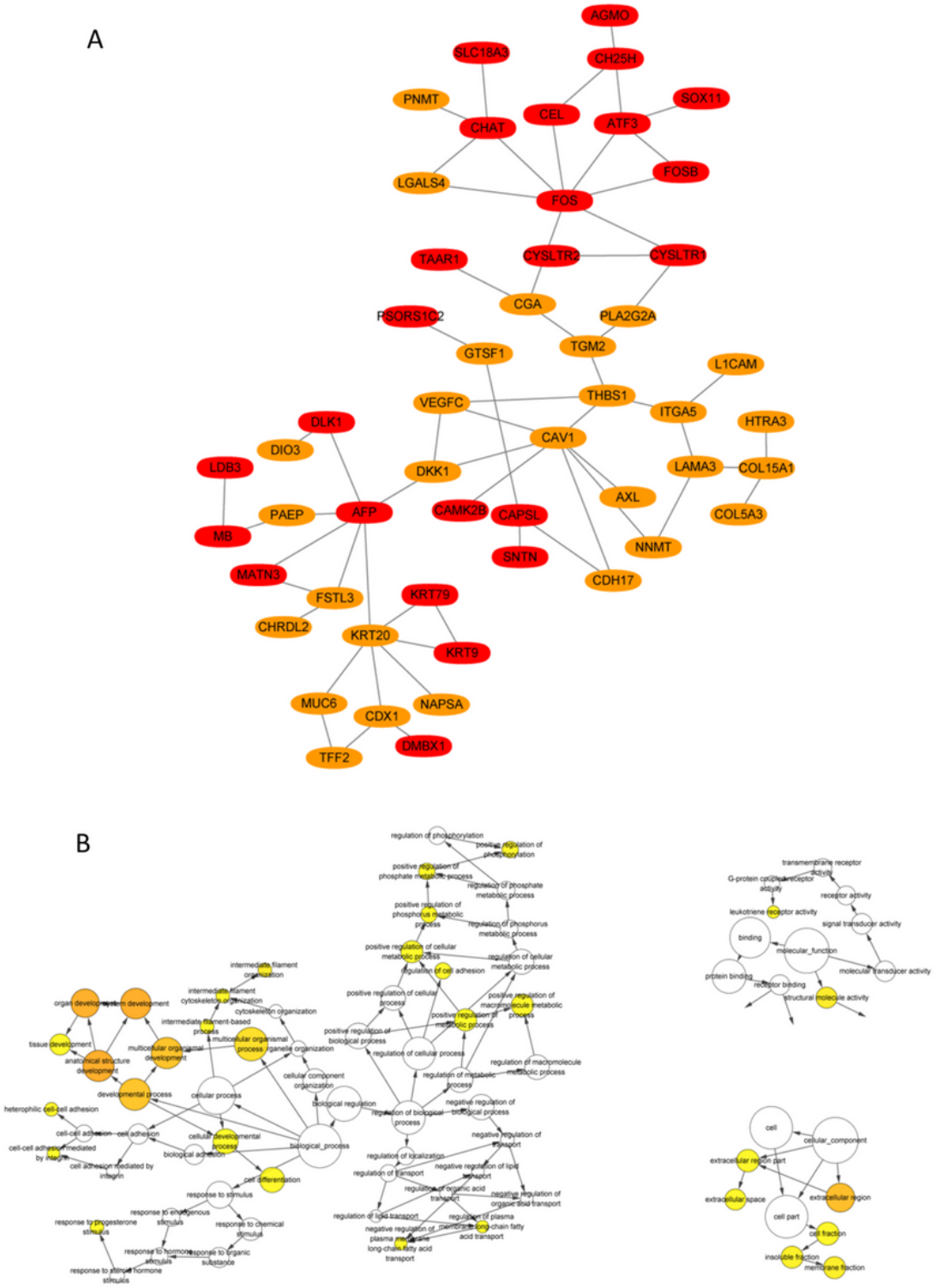

Figure 4

the interaction network of DEGs. (A) PPI network of DEGs, the network had 74 nodes (genes) and 72 edges, the red circles represented up-regulated genes, the blue circles represented down-regulated genes. (B) the KEGG pathway of DEGs, The depth of the color represented the degree of pathway enrichment. (C) the molecular function of DEGs, the depth of the color represented the degree of molecular function

enrichment. (D) the Cell component of DEGs, the depth of the color represented the degree of cell component.

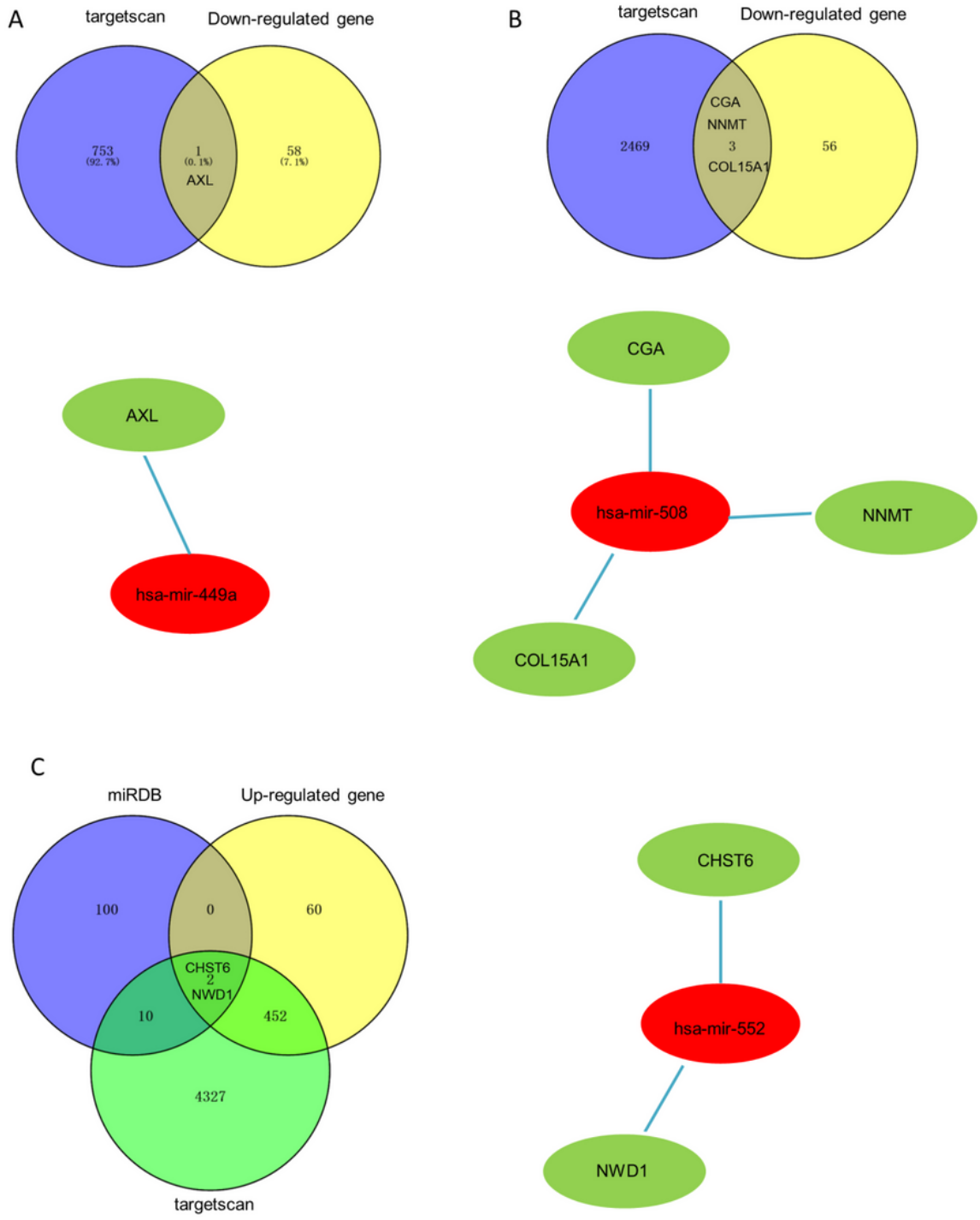

Figure 5

The DEGs intersected the targets of validated miRNAs. (A) hsa-mir-449a. (B) hsa-mir-552. (C) hsa-mir-552.

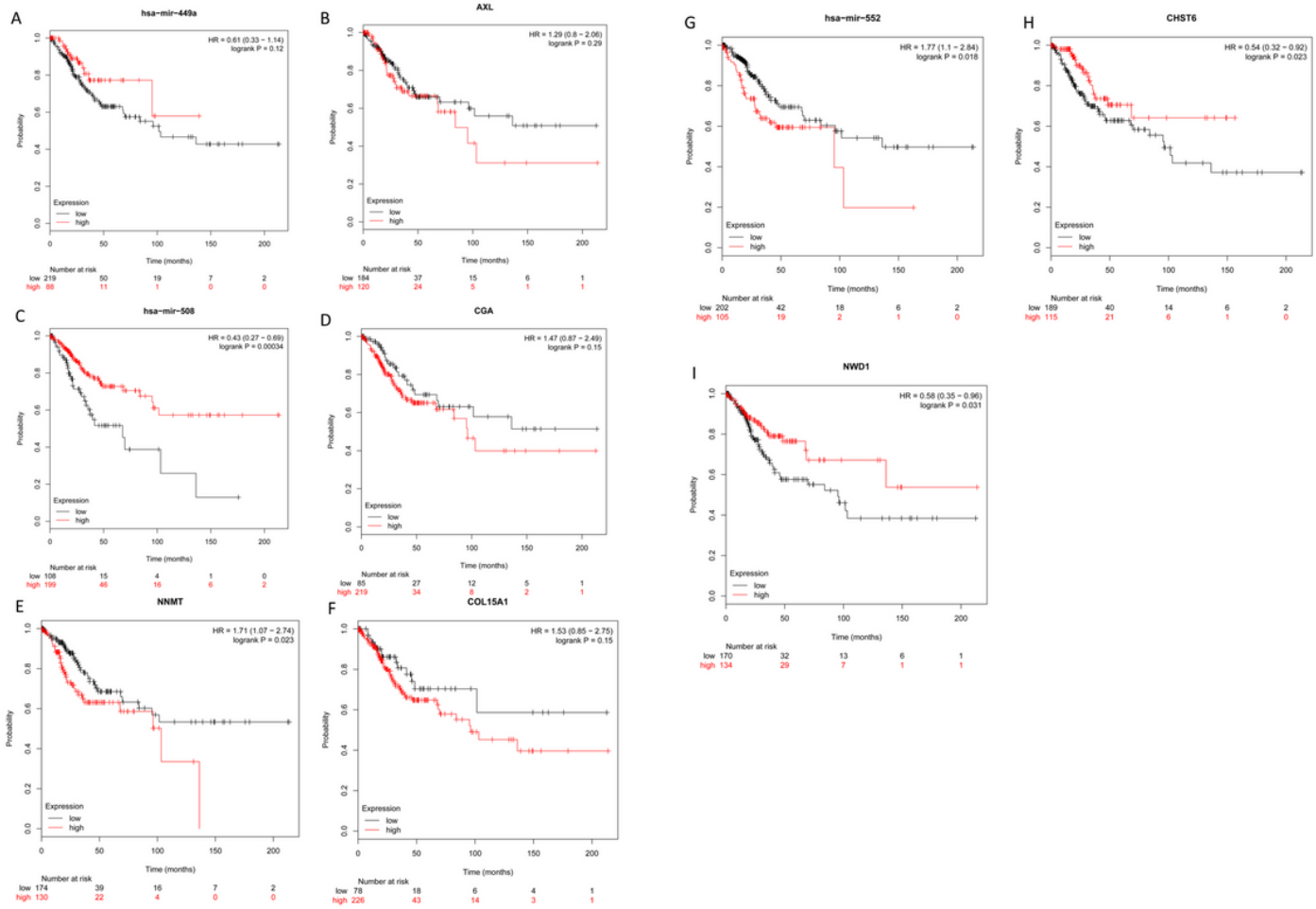

Figure 6

The prognostic value of miRNAs and their targets in cervical cancer patients. (A) Prognostic value of hsa-mir-449a. (B) Prognostic value of AXL, the target of hsa-mir-449a. (C)Prognostic value of hsa-mir-508. (D) Prognostic value of CGA, the target of hsa-mir-508. (E) Prognostic value of NNMT, the target of hsa-mir-508. (F) Prognostic value of COL15A1, the target of hsa-mir-508. (G) Prognostic value of hsa-mir-552. (H) Prognostic value of CHST6, the target of hsa-mir-449a. (I) Prognostic value of NWD1, the target of hsa-mir-449a.

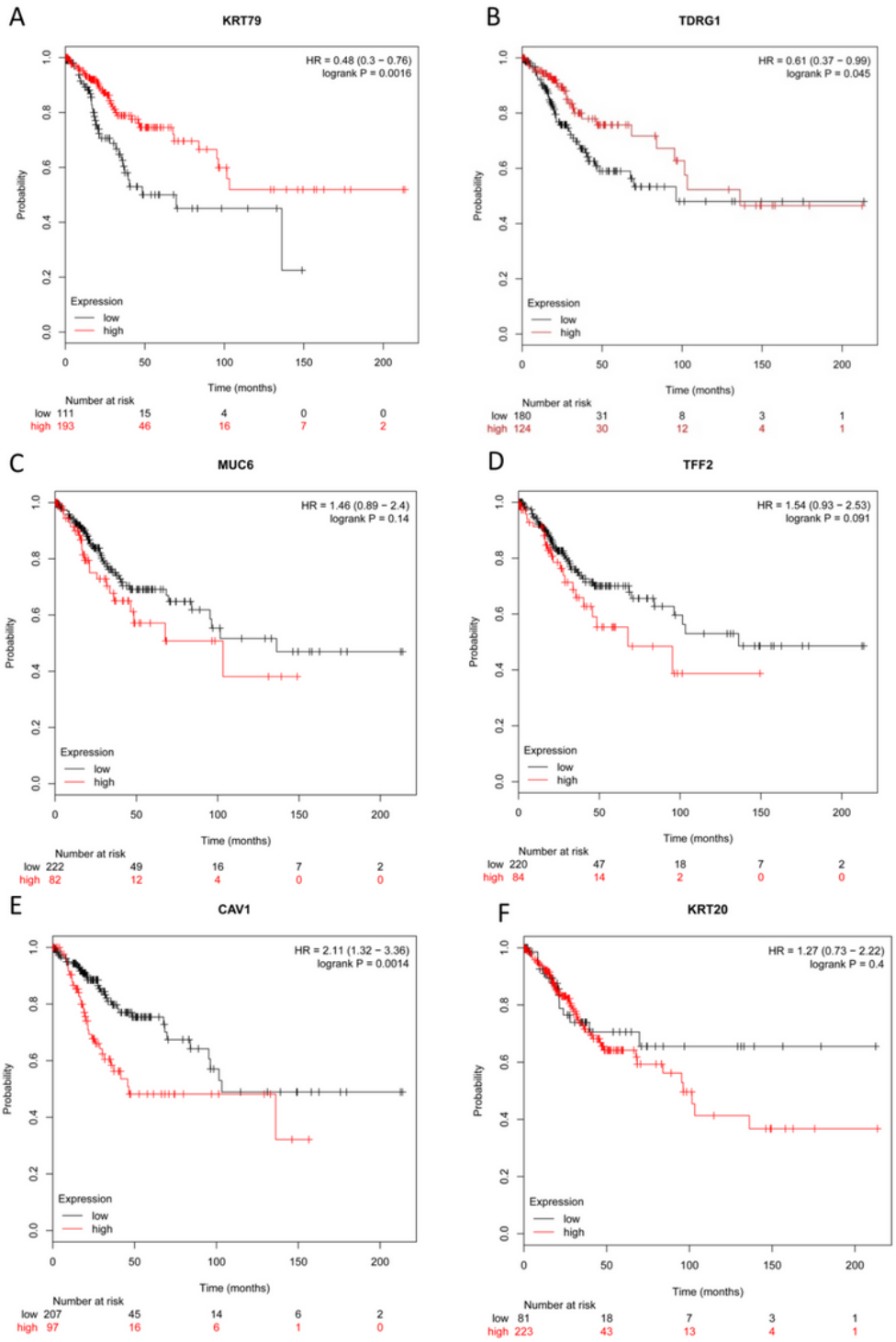

Figure 7

The prognostic value of key DEGs in cervical cancer patients. (A) Prognostic value of up-regulated gene KRT79. (B) Prognostic value of up-regulated gene TDRG1.(C) Prognostic value of down-regulated gene MUC6. (D) Prognostic value of down-regulated gene TFF2. (D) Prognostic value of key node gene CAV1 of PPI network. (E) Prognostic value of key node gene KRT20 of PPI network.