Multi-Omics Integrative Analysis Identifying *EPC1* as a Prognostic Biomarker in Head and Neck Squamous Cell Carcinoma

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Research

Keywords: EPC1, head and neck squamous cell carcinoma, multi-omics, database, prognosis

Posted Date: May 24th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-507801/v1

Abstract

Background: To investigate the prognostic significance and related mechanisms of the expression of enhancer of polycomb homolog 1 (EPC1) in head and neck squamous cell carcinoma (HNSCC) from a multi-omics perspective.

Methods: The Kaplan-Meier plotter was used to evaluate the prognostic significance of EPC1 . Based on the LinkedOmics, UALCAN, and Timer platforms, the multi-omics expression of EPC1 in HNSCC was explored to investigate mechanisms affecting prognoses.

Results: At the genetic level, 8208 genes were negatively correlated with EPC1 expression, and 11,956 genes were positively correlated with EPC1 expression. For the noncoding region, a competing endogenous RNA (ceRNA) network was constructed, and 6 microRNAs (miRNAs) and 3 long noncoding RNAs (lncRNAs) were identified. At the protein level, a protein-protein interaction (PPI) network related to EPC1 expression was constructed and was involved in human papillomavirus (HPV) infection, endocrine resistance, and multiple cancer pathways. At the immune level, EPC1 expression was correlated with a variety of immune cells, immune molecules, and chemokine receptors, which together constitute the immune microenvironment of tumors. According to the clinical data, high EPC1 expression in HNSCC was a predictor of patient prognosis (hazard ratio (HR)=0.64; 95% confidence interval (CI) 0.49-0.83; P<0.01). EPC1 expression differentiated clinical subtypes and was related to key factors such as TP53 and HPV (P<0.05).

Conclusion: High EPC1 expression is a protective factor in HNSCC and benefits patient survival. EPC1 may participate in the genomics, transcriptomics, proteomics, and immunomics of HNSCC, and the results can provide a reference for the development of targeted drugs and the evaluation of patient prognosis.

Background

Due to increases in tobacco use and the human papillomavirus (HPV) infection rate, the number of patients with head and neck squamous cell carcinoma (HNSCC) is increasing, which is one of the most common cancers and accounts for approximately 5% of all malignancies[1, 2]. Successful development of targeted therapies in biomarker-selected patients for personalized medicine has shifted expectations in cancer research[3, 4], but the lack of targetable genomic abnormalities in HNSCC has limited the development of targeted therapies in the past[5]. Thus, identifying a reliable molecular biomarker for predicting the prognosis of HNSCC patients is an urgent task. To better treat HNSCC patients, a large number of studies have focused on obtaining relevant biomarkers to predict patient prognosis[6–8]. However, because the human body is a complex organism and the occurrence and development of cancer involve many aspects, the limitations of mining disease-related factors based on the one-omics perspective have become increasingly apparent in recent years. Additionally, databases that are being constantly improved provide technical support, enabling multi-omics studies involving genomics,

transcriptomics, and proteomics. Therefore, the application of multi-omics data systems to explore the targets of cancer biomarkers has become an important trend in precision medicine, allowing joint research on diseases from macro and micro aspects[9].

Enhancer of polycomb homolog 1 (*EPC1*) has a protective function against DNA damage. Epigenetic factor *EPC1* is a master regulator of the DNA damage response by interacting with *E2F1* to silence death and activate metastasis-related gene signatures[10]. To date, pathways known to be associated with this gene include chromatin-modifying enzymes, chromatin organization, and histone acetyl transferases (HATs)[11]. Sophisticated studies have demonstrated that *EPC1* is involved in constituting the NuA4 HAT complex, and the crystal structure and molecular basis for EPC1 bound to MBTD1 were determined[12]. Additionally, hsa_circ_0007919 knockdown can yield hsa-let-7a to downregulate *EPC1* mRNA[13]. According to literature reports, abnormal *EPC1* expression is present in both endometrial stromal sarcoma[14, 15] and ossifying fibromyxoid tumors[16], whereas *EPC1* silencing inhibits lung cancer cell proliferation and tumor growth[17]. Additionally, the *EPC1* has shown correlations with patient prognosis in microarray screenings of nasopharyngeal cancer[18]. All these data suggest that *EPC1* is a prognostic biomarker worth studying. Therefore, we aim to provide further insight into the prognostic significance of *EPC1* in patients with HNSCC and to comprehensively analyze the *EPC1* from a multi-omics perspective to explore its mechanism of action.

Results

Effect of the differential expression of EPC1 on the prognosis and clinical outcomes of patients with HNSCC

In the TISIDB platform, Spearman correlation analysis was performed to study the associations of *EPC1* expression with HNSCC subtypes. *EPC1* expression levels were not equal or completely equal between different subtypes (Fig. 1A). The Kaplan-Meier plotter platform was used to analyze survival and *EPC1* expression (Fig. 1B). The median survival time for the low-*EPC1* expression group was 33.10 months, and the median survival time for the high-*EPC1* expression group was 61.27 months; the difference was statistically significant (HR < 1, P < 0.01), suggesting that *EPC1* is a protective factor against HNSCC and that patients with high *EPC1* expression have a better prognosis.

Based on TCGA samples and the UALCAN website, *EPC1* expression in HPV-positive HNSCC tumors was not only significantly higher than that in paracancerous tissues (P < 0.01) but also significantly higher than that in HPV-negative HNSCC samples (P < 0.01) (Fig. 1C). Using HPV-positive HNSCC samples, we further explored the relationship between *EPC1* expression and patient prognosis. The results showed that patient prognosis was significantly better with higher *EPC1* expression (Fig. 1D) (P < 0.01). However, no significant effect of *EPC1* expression on patient prognosis was found when analyzing HPV-negative HNSCC samples. In addition, compared with that in TP53-mutated HNSCC, *EPC1* expression in wild-type TP53 HNSCC was significantly higher (P < 0.05) (Fig. 1E). In HPV-positive HNSCC samples, the expression of wild-type TP53 *EPC1* was relatively higher. In HPV-negative HNSCC samples, no significant difference

in *EPC1* expression was identified between wild-type TP53 and TP53-mutated samples (Fig. 1F). Therefore, we hypothesize that *EPC1* and TP53 may be correlated.

Screening and functional prediction of genes associated with the differential expression of EPC1 in HNSCC

LinkedOmics was used to screen genes that were significantly positively correlated with the *EPC1* gene and genes that were significantly negatively correlated with the *EPC1* gene. A total of 20,164 related genes were obtained, including 8208 genes with negative correlations and 11,956 genes with positive correlations, and volcano plots were drawn (Fig. 2A). The notable positively correlated genes included *ZNF41, NR2C2*, and *CEP350*. The notable negatively correlated genes included *MRPL28, C14orf156* and *TMEM280*. After obtaining the gene dataset, we further performed GSEA. The rank criteria were a P-value < 0.05, an FDR ≤ 0.05, a minimum number of genes (size) = 5, and simulations = 500. KEGG pathway analysis was conducted. We selected “Redundancy reduction: Weighted set cover” and screened 5 positively correlated KEGG pathways (labeled blue, Fig. 2B): phosphatidylinositol signaling system, cell adhesion molecules, cGMP-PKG signaling pathway, Rap1 signaling pathway, and pathways in cancer. We also screened 5 negatively correlated KEGG pathways (labeled orange, Fig. 2B): purine metabolism, thermogenesis, spliceosome, proteasome, and ribosome. Using pathways in cancer as an example, a total of 190 genes were enriched (enrichment score = 0.58; normalized enrichment score = 1.56; P < 0.01), and the difference was statistically significant (Fig. 2C). The above steps were repeated for the GO analysis (biological process). Five positively correlated biological processes were screened (labeled blue, Fig. 2D): protein autophosphorylation, covalent chromatin modification, regulation of GTPase activity, positive regulation of cell motility, and Ras protein signal transduction. Additionally, 5 negatively correlated biological processes were screened (labeled orange, Fig. 2D): protein folding, nucleoside triphosphate metabolic process, protein targeting, ribonucleoprotein complex biogenesis, and mitochondrial gene expression. The above analyses showed that the differential expression of *EPC1* at the gene level is related to cancer pathways. *EPC1* may regulate the metastasis and spread of tumor cells by positively enhancing the function of cell adhesion molecules, regulating cell migration, and reducing the expression of mitochondrial genes, thereby improving patient prognosis.

Construction of a lncRNA-miRNA-mRNA network based on the differential expression of EPC1 in HNSCC samples

A total of 52 *EPC1* gene-related miRNAs that were experimentally verified were identified using the TarBase V. 8 database, and 201 miRNAs differentially expressed in HNSCC were identified using the YM500v2 platform. The two datasets were intersected, resulting in 10 overlapping miRNAs (Fig. 3A). The LncBase v.2 database was used to predict the related lncRNAs for the 10 miRNAs, and the Lnc2Cancer database was used to obtain experimentally verified HNSCC-related lncRNAs; 27 lncRNAs differentially expressed between head and neck cancer and HNSCC were identified, and 6 lncRNAs overlapped between the 2 (Fig. 3B). From 6 lncRNAs and 10 miRNAs whose associations had been experimentally verified, 3 lncRNAs were screened (ENSG00000130600 (H19), ENSG00000234741 (GAS5), and ENSG00000205592

(MUC19)), and 7 miRNAs were screened (hsa-miR-26a-5p, hsa-miR-26b-5p, hsa-miR-454-3p, hsa-miR-130b-3p, hsa-miR-301a-3p, hsa-miR-182-5p, and hsa-miR-101-3p). Highcharts software was used to construct lncRNA-miRNA-mRNA network diagrams, with the thickness of the line widths indicating the degree of possible association between nodes (Fig. 3C).

Screening and pathway enrichment of proteins related to differential EPC1 expression in HNSCC

LinkedOmics was used to screen 13 genes that were positively related to *EPC1* gene expression (Fig. 4A) and 7 proteins that were negatively related to *EPC1* expression (Fig. 4B), all satisfying $P < 0.05$. Corresponding heat maps were drawn. Using the STRING database, an interaction network consisting of 21 proteins was constructed (Fig. 4C), and protein enrichment analysis was used to obtain the top 10 related pathways in terms of gene ratios (Fig. 4D). The PPI network suggested that proteins co-expressed with *EPC1* may be involved in various cancer-related signaling pathways such as HPV infection, endocrine resistance, cell cycle disruption, plaque adhesion, breast cancer, gastric cancer, hepatocellular carcinoma, pancreatic cancer, and small cell lung cancer.

Differential expression of EPC1 among all HNSCC samples, HPV-positive HNSCC samples, and HPV-negative HNSCC samples and the association with immunity

Immune molecules associated with *EPC1* expression were screened using the TISIDB platform. *EPC1* expression in HNSCC samples was positively correlated with the immune enhancer *TNFSF15* and two chemokine receptors *CCR4* and *CCR8*, and high expression of these three proteins was predictive of the prognosis of patients with HPV-positive head and neck cancer; the difference was statistically significant (Fig. 5).

At the cellular level, the HNSCC samples, HPV-positive HNSCC samples, and HPV-negative HNSCC samples were subjected to immunological analysis using the Timer platform. Data from EPIC, CIBERSORT, XCELL, and other tool websites were integrated and used in the analysis. After adjusting for tumor purity, 12 EPC1-related immune cells were screened out from the HNSCC samples with the restriction condition that all three sample types reached $|rho|>0.3$ and $P < 0.05$ (Table 1). These results suggest that *EPC1* may alter the tumor microenvironment of HNSCC by affecting the expression of immune-related molecules and immune proteins.

Table 1
Correlation between *EPC1* expression and the level of immune infiltration

CELL TYPE	HNSCC (n = 522)	HNSCC-HPV^{-} (n = 422)	HNSCC-HPV^{+} (n = 98)
B cell	0.460	0.440	0.442
Endothelial cell	0.430	0.436	0.450
Mast cell	0.441	0.324	0.345
Myeloid dendritic cell	0.429	0.429	0.368
Neutrophil	0.387	0.397	0.424
NK cell	0.403	0.392	0.352
T cell CD4+	0.424	0.387	0.596
T cell CD4+ (nonregulatory)	0.361	0.302	0.521
T cell CD4 + memory resting	0.360	0.329	0.519
T cell CD4 + Th1	-0.349	-0.362	-0.417
T cell CD8+	-0.324	-0.307	-0.361
T cell regulatory (Tregs)	0.547	0.495	0.682

Discussion

EPC1 is a multi-comb homologue 1 (*Drosophila*) enhancer involved in the regulation of cell growth and transcription[19]. *EPC1* anomalies may be involved in ossifying fibromyxoid tumors[16], endometrial stromal sarcoma[20], pancreatic cancer[21], and other cancers. However, the effect of this gene on the survival prognosis of patients with HNSCC has not been studied. The survival time for HNSCC patients with high *EPC1* expression is long, suggesting that *EPC1* may have beneficial effects on the survival prognosis of HNSCC patients, an observation that was not noted in previous studies. The results of this study showed that *EPC1* expression varied in different HNSCC subtypes and that high *EPC1* expression was conducive to prolonging the survival time of HNSCC patients. In HPV-positive HNSCC samples, *EPC1* expression was relatively high, indicating that high *EPC1* expression is a protective factor. In addition, among the *EPC1*-related protein enrichment pathways, HPV infection-related pathways predominate, suggesting that *EPC1* and HPV may have a certain correlation that affects patient prognosis. In addition, TP53 mutations often indicated a poor prognosis, and *EPC1* expression was relatively low in all HNSCC samples and HPV-positive HNSCC samples with TP53 mutations. *EPC1* expression was relatively high in wild-type TP53 samples, suggesting that high *EPC1* expression may improve the prognosis of HNSCC patients.

To further investigate the possible mechanism by which *EPC1* affects the prognosis of HNSCC, we performed multi-omics analysis of *EPC1*. At the gene level, *EPC1*-related gene enrichment results indicated that *EPC1* participates in biological processes such as cancer-related pathways, cell adhesion, and cell mobility. In transcriptomics studies, lncRNA H19 is an important link in the *EPC1*-related lncRNA-miRNA-mRNA network. High lncRNA H19 expression is positively correlated with the growth, migration, and invasion of lung tumor cells. H19 may interact with miR-200a, leading to a poor patient prognosis [22]. Low H19 expression is associated with a poor prognosis for patients with microinvasive follicular thyroid carcinoma and can be used to predict distant metastasis[23]. These results suggest that *EPC1* may affect the prognosis of patients with HNSCC through lncRNA H19, leading to different prognoses in different cancer patients. In proteomics studies, *EPC1*-related proteins are mainly involved in HPV infection, endocrine resistance, the cell cycle, and a variety of cancer pathways. In this study, high *EPC1* expression in HPV-positive HNSCC samples had a significant positive effect on prognosis, suggesting that *EPC1* and HPV may be associated with the survival prognosis of patients. At the immunomics level, intratumoral immune status is a key factor affecting the patient survival rate and the response to immunotherapy. The tumor microenvironment has certain clinical significance in predicting therapeutic effects[24]. Three proteins(*CCR4, CCR8* and *TNFSF15*)were positively correlated with *EPC1*, and high expression of these 3 proteins predicted relative prolongation of patients' survival time. Furthermore, *EPC1*-related immune cells, such as CD4 and CD8 T cells, play a key role in controlling tumor growth. Immune molecules such as *CCR4, CCR8* and *TNFSF15* together with immune cells constitute the tumor microenvironment, affecting the prognosis of patients.

Conclusions

This study did not investigate the role of *EPC1* alone but rather investigated the differentially expressed genes, ceRNA networks, interacting proteins, and immune infiltration levels associated with *EPC1* in HNSCC samples to fully consider the linkage of *EPC1* from points to networks. The data used in this study were experimentally validated or genetically sequenced based on real-world data. Using two databases, we further confirmed that high *EPC1* expression is a favorable factor for the prognosis of patients with HNSCC. However, using the *EPC1* gene as a biomarker to predict the prognosis of patients with HNSCC has not yet been clinically validated and should be further investigated in future studies.

This study has some limitations. In all cancers occurring in the head and neck region, including the oral cavity, oropharynx, hypopharynx, and larynx, gene expression among subsites may differ. The data included in this study are from public databases. Most of the data do not distinguish HNSCC subsites, and we must distinguish different subsites in further studies.

Materials And Methods

Data source

Only patients with HNSCC and RNA sequencing data uploaded to The Cancer Genome Atlas (TCGA) database (https://www.tcga.org/) were included in this study. The clinical data and gene expression profiles of HNSCC patients were downloaded from Genomic Data Commons (https://portal.gdc.cancer.gov/). Clinical data were mainly used for survival analysis, and gene expression profiles were used for subsequent multi-omics analysis. This study was conducted with reference to TCGA publication guidelines.

Study design

The associations between gene expression and key prognostic factors

To analyze possible associations between clinical parameters and *EPC1* expression, the TISIDB database (http://cis.hku.hk/TISIDB/), which integrates clinical data from TCGA, was used to identify differences in *EPC1* expression between different HNSCC subtypes[25]. Survival curves associated with *EPC1* expression in HNSCC were plotted using the Kaplan-Meier plotter (http://kmplot.com/analysis/) [26]. Overall survival (OS) was selected for the HNSCC clinical end point analysis. OS is defined as the period from the date of diagnosis to the date of death from any cause[27]. UALCAN (http://ualcan.path.uab.edu/) is a web platform constructed based on JavaScript, CSS, and PERL-CGI. Differences in *EPC1* expression in HNSCC can be visualized by using UALCAN according to TP53 mutation status or the presence of HPV[28]. Timer 2.0 (http://timer.comp-genomics.org/) was used to analyze 3 sample types: total HNSCC samples, HPV-positive HNSCC samples, and HPV-negative HNSCC samples. Therefore, the association between the *EPC1* and clinical outcomes of HNSCC patients was investigated based on the presence of HPV[29]. At the same time, the Wilcoxon rank sum test was used to analyze the relationship between the *EPC1* and TP53 mutation status.

Screening and functional enrichment of EPC1 expression-related genes

LinkedOmics (http://www.linkedomics.org/login.php) includes multi-omics data, which were used to screen differentially expressed genes related to *EPC1* in HNSCC. The Spearman correlation test was used to find *EPC1* association results. Volcano plots were visualized using the “chart-studio” package of Python. Then, we performed a gene set enrichment analysis (GSEA). The rank criteria were a P-value < 0.05, a false discovery rate (FDR) ≤ 0.05, a minimum number of genes (size) = 5, and simulations = 500. Additionally, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed. This methodology was used to explore the mechanism by which *EPC1* protects against HNSCC at the gene expression level[30].

Construction of a competing endogenous RNA (ceRNA) network

To explore the interaction of *EPC1* with long noncoding RNAs (lncRNAs) and microRNA (miRNA), DIANA-tools and other databases were used to construct a CeRNA network. DIANA-tools contains the TarBase v.8 and LncBase v.2 databases. TarBase v.8 (http://www.microrna.gr/tarbase) is a database of miRNA-gene interactions that have been confirmed by experiments [31]. TarBase v.8 and the YM500v2 miR-Seq database (http://ngs.ym.edu.tw/ym500v2/index.php) were used to screen *EPC1* gene-related miRNAs in HNSCC samples [32]. LncBase v.2 (www.microrna.gr/LncBase) was used as a primary screening tool to determine the relationship between *EPC1*-related miRNAs and lncRNAs[33]. The Lnc2Cancer (http://www.bio-bigdata.net/lnc2cancer/) database was used to obtain experimentally validated HNSCC-related lncRNAs[34]. Then, LncBase v.2 and Lnc2Cancer were together used to determine the lncRNAs. Finally, the intersection of data from different sources was used to construct a Venn diagram using Edraw software (https://www.edrawsoft.cn/edraw/). miRNAs and lncRNAs were screened out, and a lncRNA-miRNA-mRNA Sankey diagram was plotted using Highcharts software (http://www.highcharts.com/).

Protein-protein interaction (PPI) networks and KEGG pathway enrichment

To reveal the effect of *EPC1* in proteomics, *EPC1*-related proteins in HNSCC were screened using LinkedOmics (set at $P < 0.05$) and were used to construct *EPC1*-related PPI networks with the help of the STRING database[35] (https://string-db.org/). In addition, KEGG pathway enrichment was used to predict the role of *EPC1* at the protein level in HNSCC, which was visualized using the “ggplot2” package of R software.

EPC1 -related immune cells and immunoreactive substances in HNSCC samples

Timer 2.0 (http://timer.comp-genomics.org/) was used to systematically analyze immune infiltration. The tool integrates 5 websites (EPIC[36], QUANTISEQ[37], MCP-COUNTER[38], XCELL[39], and CIBERSORT[40]) and applies its own existing data[41]. The associations between immune infiltration and *EPC1* gene expression and between immune cells and clinical outcomes in HNSCC patients in 3 types of samples (all HNSCC, HPV-positive HNSCC, and HPV-negative HNSCC) were explored. The TISIDB platform (http://cis.hku.hk/TISIDB/) can also be used to obtain data regarding the differential expression of *EPC1*-related genes, immune molecules, and chemokine receptors in HNSCC. A survival curve and scatter plot were visualized to reveal the possible mechanism of this gene in immunology.

Statistical analysis

Kaplan-Meier curves were used to compare survival time differences. OS was selected for the HNSCC clinical end point analysis. Hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs) were calculated to assess the role of EPC1. A log-rank test $P < 0.05$ indicates a significant survival time difference. The results were verified by another database[36]. We also performed GSEA. The rank criteria

were a P-value < 0.05 and an FDR ≤ 0.05. Additionally, Spearman correlation analysis and the Wilcoxon rank sum test were also applied to show the correlations between the EPC1 gene and other factors in accordance with specific conditions.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Fujian Provincial Hospital.

Consent for publication

Written informed consent was obtained from the patients.

Competing interests

None.

Funding

This work was supported by the Funding project of Fujian Medical University College Student Innovation and Entrepreneurship Training Program (Grant No. C19071).

Authors' contributions

DYM wrote the manuscript; CH and CTJ revised the manuscript; CWH and HJP did the data analysis and data collection.

Availability of data and material

All the data were available upon appropriate request.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel R, Torre L, Jemal A: **Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries**. *CA: a cancer journal for clinicians* 2018, **68**(6):394-424.
2. Marur S, Forastiere A: **Head and Neck Squamous Cell Carcinoma: Update on Epidemiology, Diagnosis, and Treatment**. *Mayo Clinic proceedings* 2016, **91**(3):386-396.

3. Dai Y, Zhang Y, Yang M, Zhou L, Pan H, Xiao T, Yuan L, Wu Y, Chen M, Chen L *et al*: **Radiosensitivity-Related Genes and Clinical Characteristics of Nasopharyngeal Carcinoma**. *BioMed research international* 2020, **2020**:1705867.
4. Torres-Ayuso P, An E, Nyswaner K, Bensen R, Ritt D, Specht S, Das S, Andresson T, Cachau R, Liang R *et al*: **TNIK is a therapeutic target in Lung Squamous Cell Carcinoma and regulates FAK activation through Merlin**. *Cancer discovery* 2021.
5. **Comprehensive genomic characterization of head and neck squamous cell carcinomas**. *Nature* 2015, **517**(7536):576-582.
6. Zhu Y, Cao X, Zhang X, Chen Q, Wen L, Wang P: **DNA methylation-mediated Klotho silencing is an independent prognostic biomarker of head and neck squamous carcinoma**. *Cancer management and research* 2019, **11**:1383-1390.
7. Yazdani J, Ghavimi M, Jabbari Hagh E, Ahmadpour F: **The Role of E-Cadherin as a Prognostic Biomarker in Head and Neck Squamous Carcinoma: A Systematic Review and Meta-Analysis**. *Molecular diagnosis & therapy* 2018, **22**(5):523-535.
8. Cho J, Lim Y: **Prognostic impact of regulatory T cell in head and neck squamous cell carcinoma: A systematic review and meta-analysis**. *Oral oncology* 2021, **112**:105084.
9. Dhungana S, Molloy B, Plumb R, Li J: **High Throughput Targeted Metabolomics Panels to Support Large Studies**. *Journal of biomolecular techniques : JBT* 2020, **31**:S20-S21.
10. Wang Y, Alla V, Goody D, Gupta S, Spitschak A, Wolkenhauer O, Pützer B, Engelmann D: **Epigenetic factor EPC1 is a master regulator of DNA damage response by interacting with E2F1 to silence death and activate metastasis-related gene signatures**. *Nucleic acids research* 2016, **44**(1):117-133.
11. Fink D, Yau T, Nabbi A, Wagner B, Wagner C, Hu S, Lang V, Handschuh S, Riabowol K, Rülicke T: **Loss of Ing3 Expression Results in Growth Retardation and Embryonic Death**. *Cancers* 2019, **12**(1).
12. Zhang H, Devoucoux M, Song X, Li L, Ayaz G, Cheng H, Tempel W, Dong C, Loppnau P, Côté J *et al*: **Structural Basis for EPC1-Mediated Recruitment of MBTD1 into the NuA4/TIP60 Acetyltransferase Complex**. *Cell reports* 2020, **30**(12):3996-4002.e3994.
13. Wang T, Chen N, Ren W, Liu F, Gao F, Ye L, Han Y, Zhang Y, Liu Y: **Integrated analysis of circRNAs and mRNAs expression profile revealed the involvement of hsa_circ_0007919 in the pathogenesis of ulcerative colitis**. *Journal of gastroenterology* 2019, **54**(9):804-818.
14. Dickson B, Lum A, Swanson D, Bernardini M, Colgan T, Shaw P, Yip S, Lee C: **Novel EPC1 gene fusions in endometrial stromal sarcoma**. *Genes, chromosomes & cancer* 2018, **57**(11):598-603.
15. Micci F, Gorunova L, Agostini A, Johannessen L, Brunetti M, Davidson B, Heim S, Panagopoulos I: **Cytogenetic and molecular profile of endometrial stromal sarcoma**. *Genes, chromosomes & cancer* 2016, **55**(11):834-846.
16. Antonescu C, Sung Y, Chen C, Zhang L, Chen H, Singer S, Agaram N, Sboner A, Fletcher C: **Novel ZC3H7B-BCOR, MEAF6-PHF1, and EPC1-PHF1 fusions in ossifying fibromyxoid tumors–molecular characterization shows genetic overlap with endometrial stromal sarcoma**. *Genes, chromosomes & cancer* 2014, **53**(2):183-193.

17. Che C, Zhang L, Huo J, Zhang Y: **RNA interference targeting enhancer of polycomb1 exerts anti-tumor effects in lung cancer**. *International journal of clinical and experimental pathology* 2015, **8**(1):361-367.
18. Zou Z, Liu S, Ha Y, Huang B: **Construction and Analysis of lncRNA-Mediated ceRNA Network in Nasopharyngeal Carcinoma Based on Weighted Correlation Network Analysis**. *BioMed research international* 2020, **2020**:1468980.
19. Searle, Naomi, E., Pillus, Lorraine: **Critical genomic regulation mediated by Enhancer of Polycomb**. *Current Genetics Eukaryotes with Emphasis on Yeasts Fungi Mitochondria Plastids* 2018.
20. Francesca, Micci, Marta, Brunetti, Paola, Dal, Cin, Marisa, Nucci: **Fusion of the Genes BRD8 and PHF1 in Endometrial Stromal Sarcoma**. *Genes Chromosomes & Cancer* 2017.
21. Biankin A, Waddell N, Kassahn K, Gingras M, Muthuswamy L, Johns A, Miller D, Wilson P, Patch A, Wu J *et al*: **Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes**. *Nature* 2012, **491**(7424):399-405.
22. Zhao Y, Feng C, Li Y, Ma Y, Cai R: **LncRNA H19 promotes lung cancer proliferation and metastasis by inhibiting miR-200a function**. *Molecular and cellular biochemistry* 2019, **460**:1-8.
23. Dai Y, Miao Y, Zhu Q, Gao M, Hao F: **Expression of long non-coding RNA H19 predicts distant metastasis in minimally invasive follicular thyroid carcinoma**. *Bioengineered* 2019, **10**(1):383-389.
24. Huo M, Zhang Y, Chen Z, Zhang S, Bao Y, Li T: **Tumor microenvironment characterization in head and neck cancer identifies prognostic and immunotherapeutically relevant gene signatures**. *Scientific reports* 2020, **10**(1):11163.
25. Ru B, Wong C, Tong Y, Zhong J, Zhong S, Wu W, Chu K, Wong C, Lau C, Chen I *et al*: **TISIDB: an integrated repository portal for tumor-immune system interactions**. *Bioinformatics (Oxford, England)* 2019, **35**(20):4200-4202.
26. Menyhárt O, Nagy Á, Győrffy B: **Determining consistent prognostic biomarkers of overall survival and vascular invasion in hepatocellular carcinoma**. *Royal Society open science* 2018, **5**(12):181006.
27. Liu J, Lichtenberg T, Hoadley K, Poisson L, Lazar A, Cherniack A, Kovatich A, Benz C, Levine D, Lee A *et al*: **An Integrated TCGA Pan-Cancer Clinical Data Resource to Drive High-Quality Survival Outcome Analytics**. *Cell* 2018, **173**(2):400-416.e411.
28. Chandrashekar D, Bashel B, Balasubramanya S, Creighton C, Ponce-Rodriguez I, Chakravarthi B, Varambally S: **UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses**. *Neoplasia (New York, NY)* 2017, **19**(8):649-658.
29. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B, Liu X: **TIMER2.0 for analysis of tumor-infiltrating immune cells**. *Nucleic acids research* 2020, **48**:W509-W514.
30. Vasaikar S, Straub P, Wang J, Zhang B: **LinkedOmics: analyzing multi-omics data within and across 32 cancer types**. *Nucleic acids research* 2018, **46**:D956-D963.
31. Karagkouni D, Paraskevopoulou M, Chatzopoulos S, Vlachos I, Tastsoglou S, Kanellos I, Papadimitriou D, Kavakiotis I, Maniou S, Skoufos G *et al*: **DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA-gene interactions**. *Nucleic acids research* 2018, **46**:D239-D245.

32. Cheng WC, I-Fang C, Cheng-Fong T, Huang TS, Chen CY, Wang SC, Chang TY, Sun HJ, Chao YC, Cheng CC: **YM500v2: a small RNA sequencing (smRNA-seq) database for human cancer miRNome research**. *Nucleic Acids Research* 2015(D1):D862.
33. Paraskevopoulou M, Vlachos I, Karagkouni D, Georgakilas G, Kanellos I, Vergoulis T, Zagganas K, Tsanakas P, Floros E, Dalamagas T *et al*: **DIANA-LncBase v2: indexing microRNA targets on non-coding transcripts**. *Nucleic acids research* 2016, **44**:D231-238.
34. Gao Y, Wang P, Wang Y, Ma X, Zhi H, Zhou D, Li X, Fang Y, Shen W, Xu Y: **Lnc2Cancer v2.0: updated database of experimentally supported long non-coding RNAs in human cancers**. *Nucleic Acids Research* 2018.
35. **STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets**. *Nucleic acids research* 2018.
36. Racle J, de Jonge K, Baumgaertner P, Speiser D, Gfeller D: **Simultaneous enumeration of cancer and immune cell types from bulk tumor gene expression data**. *eLife* 2017, **6**.
37. Finotello F, Mayer C, Plattner C, Laschober G, Rieder D, Hackl H, Krogsdam A, Loncova Z, Posch W, Wilflingseder D *et al*: **Molecular and pharmacological modulators of the tumor immune contexture revealed by deconvolution of RNA-seq data**. *Genome medicine* 2019, **11**(1):34.
38. Becht E, Giraldo N, Lacroix L, Buttard B, Elarouci N, Petitprez F, Selves J, Laurent-Puig P, Sautès-Fridman C, Fridman W *et al*: **Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression**. *Genome biology* 2016, **17**(1):218.
39. Aran D, Hu Z, Butte A: **xCell: digitally portraying the tissue cellular heterogeneity landscape**. *Genome biology* 2017, **18**(1):220.
40. Newman A, Liu C, Green M, Gentles A, Feng W, Xu Y, Hoang C, Diehn M, Alizadeh A: **Robust enumeration of cell subsets from tissue expression profiles**. *Nature methods* 2015, **12**(5):453-457.
41. Li B, Severson E, Pignon J, Zhao H, Li T, Novak J, Jiang P, Shen H, Aster J, Rodig S *et al*: **Comprehensive analyses of tumor immunity: implications for cancer immunotherapy**. *Genome biology* 2016, **17**(1):174.

Figures

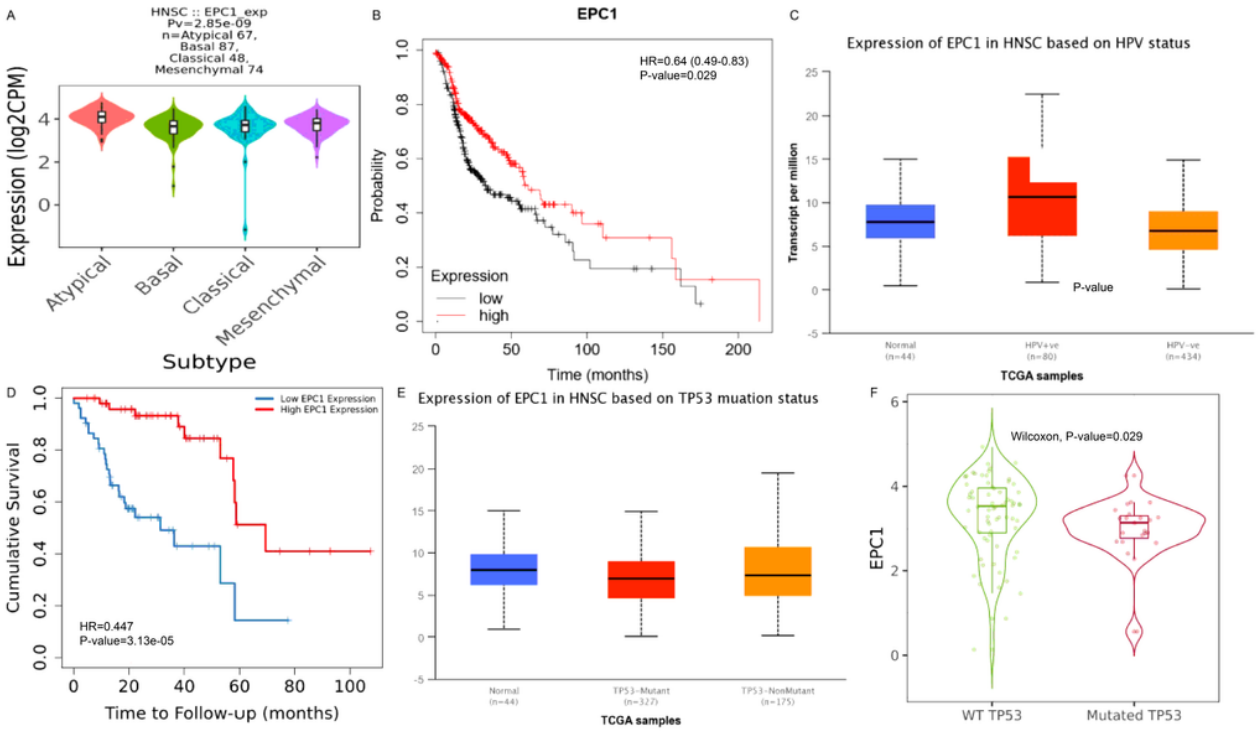

Figure 1

Correlations between the differential expression of the EPC1 gene and key prognostic factors of HNSCC (TISIDB, Kaplan-Meier plotter, UALCAN, and TIMER2.0 databases) Note: A. Expression of the EPC1 in different subtypes of head and neck tumors; B. Survival curve for EPC1 expression levels; C. Differential expression of the EPC1 in head and neck tumors based on the presence of HPV; D. Survival curve for EPC1 expression in HPV-positive head and neck cancer samples; E. Differential expression of the EPC1 in head and neck cancer based on TP53 mutation status; F. The association between the EPC1 and TP53 mutations in HPV-positive head and neck cancer samples.

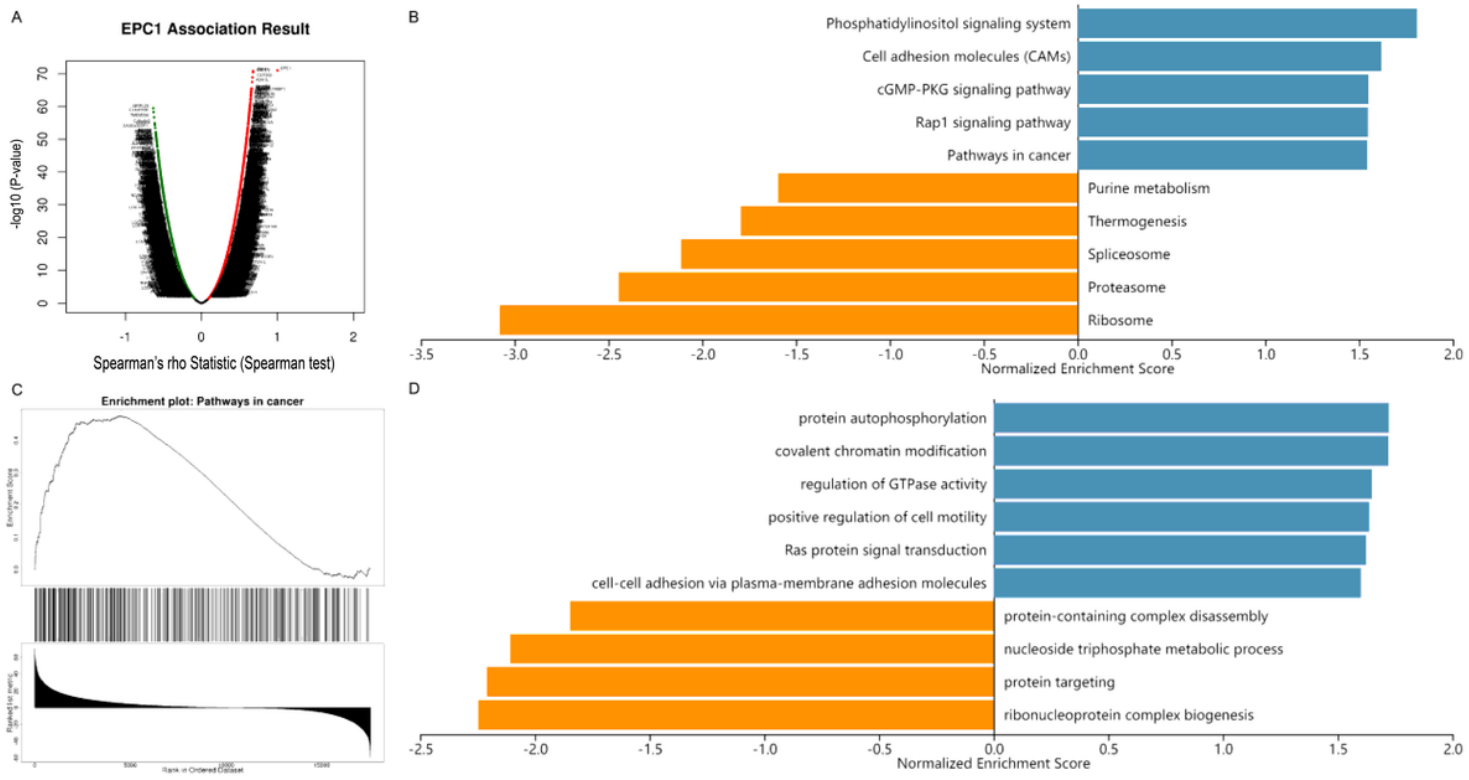

Figure 2

Screening and enrichment analysis of EPC1-related genes in HNSCC samples (LinkedOmics database) Note: A. Volcano plots for EPC1-related genes in head and neck cancer samples; B. KEGG pathway enrichment analysis of EPC1-related genes in head and neck cancer samples; C. Enrichment plots (GSEA) for pathways in cancer; D. Enrichment of EPC1-related genes in biological processes.

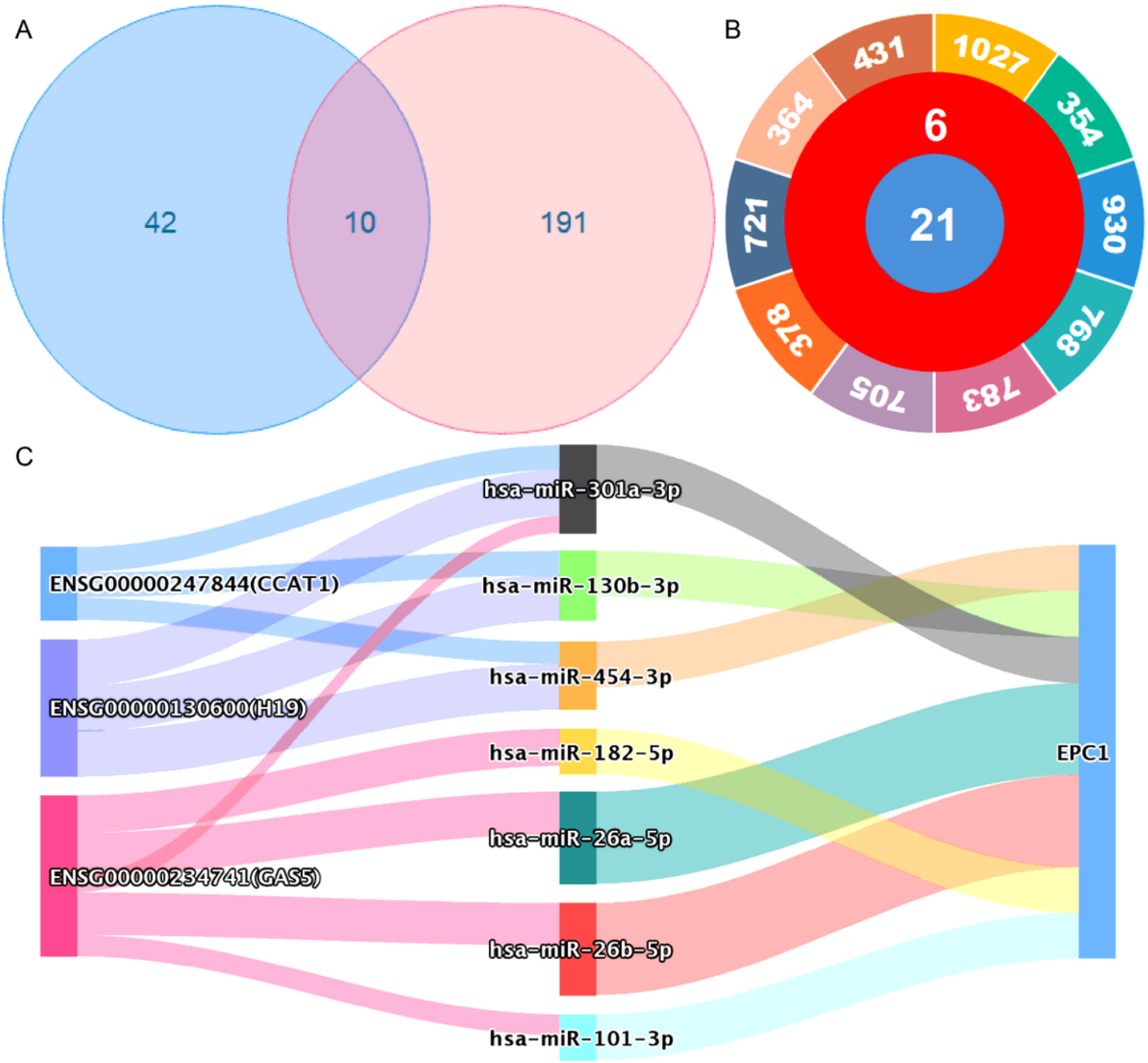

Figure 3

EPC1-related miRNAs and lncRNAs in HNSCC samples Note: A. EPC1 gene-related miRNAs in TarBase v.8 and YM500v2; B. EPC1 gene-related lncRNAs in LncBase v.2 and Lnc2Cancer; C. EPC1 gene-related lncRNA-miRNA-mRNA network diagram.

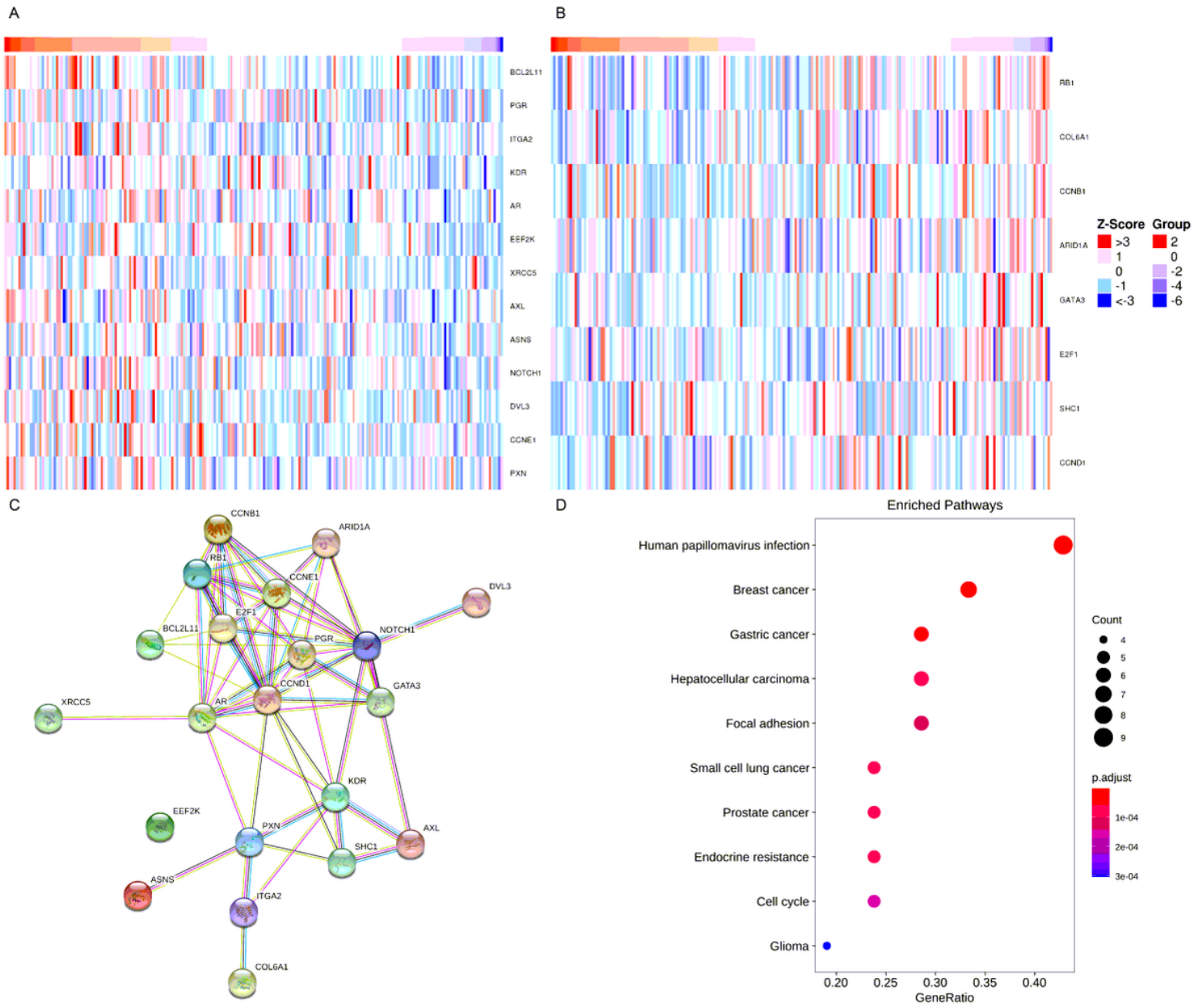

Figure 4

Proteomics study of EPC1 in HNSCC samples (LinkedOmics and STRING databases) Note: A. Proteins positively associated with EPC1 expression in head and neck cancer samples; B. Proteins negatively correlated with EPC1 expression in head and neck cancer samples; C. Protein-protein interaction network constructed from related proteins; D. Bubble diagram of pathways enriched with related proteins.

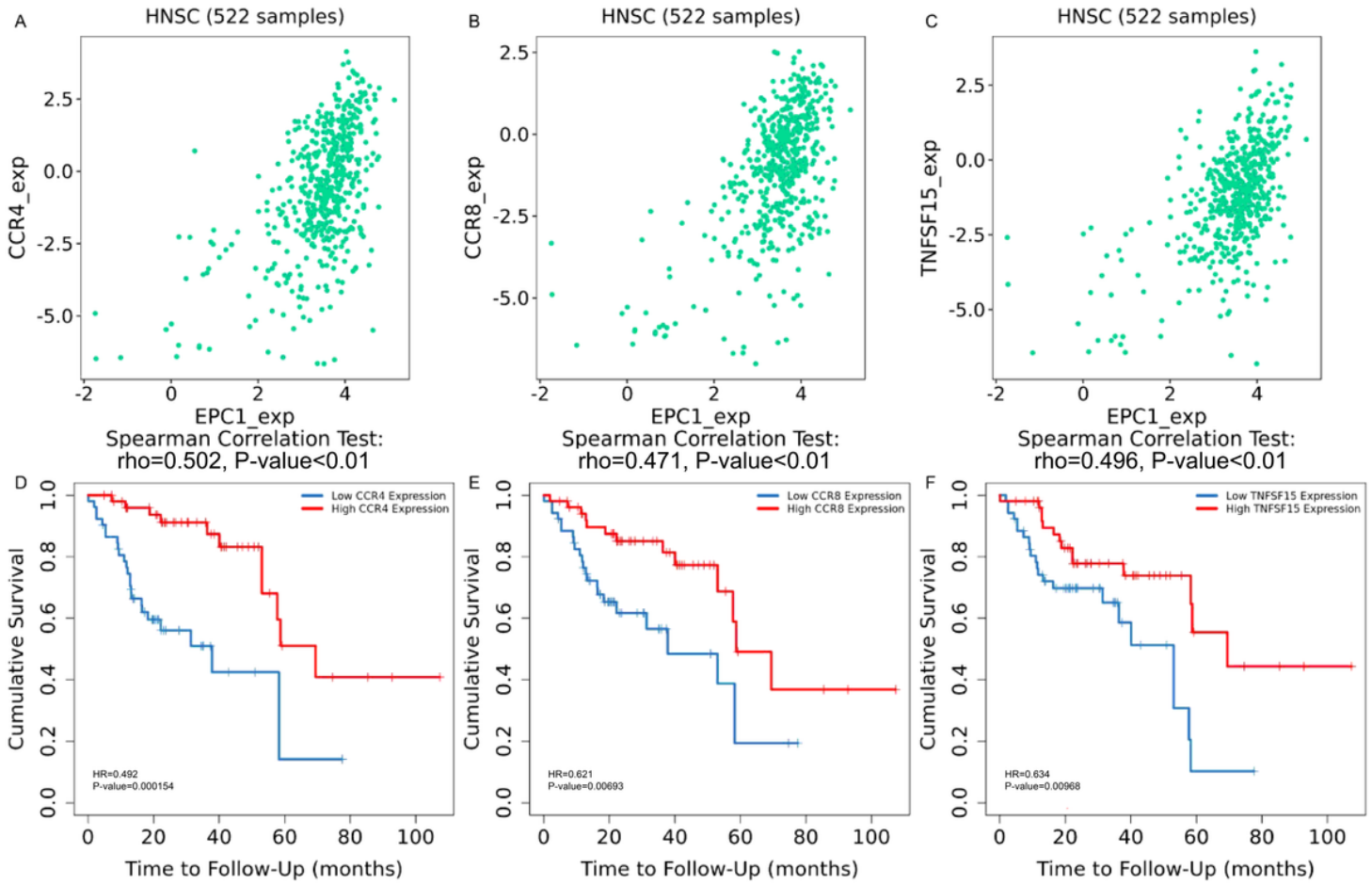

Figure 5

EPC1-related immunoreactive substances and survival curves (HNSCC samples; TISIDB and TIMER 2.0) Note: A. EPC1 was positively correlated with CCR4; B. EPC1 was positively correlated with CCR8; C. EPC1 was positively correlated with TNFSF15; D. High CCR4 expression and high overall survival (HPV-positive HNSCC samples); E. High CCR8 expression and high overall survival (HPV-positive HNSCC samples); F. High TNFSF15 expression and high overall survival (HPV-positive HNSCC samples).