Journal of Pharmaceutical and Medical Research
Print ISSN: 2663-1954
Online ISSN: 2663-1962
DOI: https://doi.org/10.61784/jpmr3038

EFFECT OF NPC1 AS AN INDEPENDENT RISK FACTOR ON THE PROGNOSIS OF CERVICAL CANCER

ZheYu Luan
Prenatal Diagnosis Center, The Sixth Affiliated Hospital of Harbin Medical University, Harbin Medical University, Harbin 150028, Heilongjiang, China.
Corresponding Email: zyluan2020@163.com

Abstract: Objective: Intracellular cholesterol transporter 1 (NPC1) plays an indispensable role in the pathological process of malignant tumor. But the value of NPC1 as a biomarker in cervical cancer has not yet been revealed. Methods: 191 cervical cancer samples with NPC1 expression data and relevant clinical characteristic information were obtained from the Cancer Genome Atlas (TCGA). A series of bioinformatics analysis methods were performed to explore the biological role of NPC1 in cervical cancer. Results: Firstly, Cox regression and the Kaplan–Meier method showed that the increased expression of NPC1 was related to the decrease of overall survival time in cervical cancer. Secondly, GO enrichment analysis disclosed that some essential physiological processes were significantly correlated with NPC1 over-expression. Thirdly, KEGG enrichment analysis indicated that cancer-related signaling pathways were correlated with NPC1 expression. Fourthly, I found the close relationship between NPC1 expression and tumor immune micro-environment of cervical cancer. Finally, four small molecule drugs with potential inhibitory effect on NPC1 expression were screened by C-Map analysis. Conclusion: The high expression of NPC1 was an independent risk factor for the poor prognosis of cervical cancer. Moreover, NPC1 might be promising biomarker for the diagnosis and treatment of cervical cancer.
Keywords: Cervical cancer; NPC1; Poor prognosis; Biomarker; Bioinformatics analysis

1 INTRODUCTION

Cervical cancer was the first most common cancer in the female reproductive system. In 2018, there were approximately 570,000 women newly diagnosed cervical cancer, and the total number of deaths has reached an incredible 311000 in worldwide [1]. The clinical outcomes of early-staged cervical cancer have greatly improved by HPV screening and vaccination strategies [2-4]. However, the prognosis of advanced cervical cancer is still unsatisfactory, whose 5-year relative survival rate is only 16.5% [5]. The poor prognosis is probably due to the shortage of effectual prediction targets in cervical cancer [6]. Consequently, it is urgent to explore new therapeutic and prognostic biomarkers to improve the prognosis of patients.

Currently, several biomarkers have been identified for the diagnosis and treatment of cervical cancer [7-8]. For example, E6 and E7 promote the malignant evolution of cervical cancer, which has been applied as a detection target in tumor screening [9]. In addition, high circGSE1 expression can predict worse overall survival (OS) and disease-free survival (DFS) [10]. Moreover, the p16/Ki67 is closely related to tumor occurrence, development and prognosis, which is of vital instructive significance for cervical cancer diagnosis [11]. Because the pathogenesis of cervical cancer was extremely complex involving a variety of gene disorders and even epigenetic regulation abnormalities. Therefore, to improve the prognosis of patients, only relying on one or several biomarkers was not enough to achieve the individualized diagnosis and treatment of cervical cancer [12]. It must be emphasized that the exploration of new biomarkers is imperative regarding a better understanding of the progression and prognosis of cervical cancer.

Intracellular cholesterol transporter 1(NPC1), also known as HGNC, encodes a transmembrane protein mediating intracellular cholesterol trafficking. NPC1 plays an essential role in maintaining lipid homeostasis [13]. It is reported that the mutation in NPC1 can be found in about 95% of Niemann-Pick type C (NPC) diseases, which caused the abnormal accumulation of cholesterol in various tissues and cells. In recent years, increasing number of studies has mentioned the role of NPC1 in promoting the malignancies progression and predicting prognosis. It has been revealed that the overexpression of NPC1 could enhance the proliferation of liver carcinoma cell, and the proliferation and migration ability was significantly decreased when NPC1 was silenced [14]. In esophageal cancer, NPC1 with promoter hypomethylation and upregulation, was associated with tumor processions such as extracellular matrix organization, cell adhesion and integrin signaling [15]. Those results suggested that NPC1 might function as an oncogene promote the cancer progression. However, the role of NPC1 in cervical cancer remains unclear to my best knowledge.

This study was the first time to comprehensively and systematically validate the high expression of NPC1 and its prognostic value in cervical cancer through big data analysis. Besides that, I revealed the underlying mechanism of NPC1 may involve in the malignant process of cervical cancer. In summary, the study will provide a new viewpoint on the mechanisms of cervical cancer pathology and a promising biomarker for prognosis and treatment.

2 PATIENTS AND METHODS

2.1 TCGA Database

The Tumor Genome Atlas (TCGA) (http://cancergenome.nih.gov/) freely provides various oncology data for researchers all over the world [16]. It contains many kinds of cancer data such as clinical characteristics, genome mutations, mRNA expression, miRNA expression, methylation data. This database has largely helped medical researchers to ameliorate the prevention, diagnosis and management of cancer. After deletion the incomplete data like lack of clinical stage or survival time, I all extract 191 cervical cancer samples for further analysis and processing. Patients' detailed clinical features, such as age, clinical stage, tummy node metastasis classification, were shown in Table 1.

Table 1 Characteristics of Patients with Cervical Cancer Based on TCGA RNA-Seq Data

Covariates	Type	Total	High	Low	P-value
Age	<=46	96(50.26%)	51(53.68%)	45(46.88%)	0.4258
	>46	95(49.74%)	44(46.32%)	51(53.12%)	
Clinical Stage	I	105(54.97%)	54(56.84%)	51(53.12%)	0.1533
	II	47(24.61%)	18(18.95%)	29(30.21%)	
	III	25(13.09%)	13(13.68%)	12(12.5%)	
	IV	14(7.33%)	10(10.53%)	4(4.17%)	
Histologic Grade	G1	11(5.76%)	6(6.32%)	5(5.21%)	0.1615
	G2	83(43.46%)	38(40%)	45(46.88%)	
	G3	78(40.84%)	37(38.95%)	41(42.71%)	
	GX	19(9.95%)	14(14.74%)	5(5.21%)	
Pathologic M	M0	93(48.69%)	44(46.32%)	49(51.04%)	0.7113
	M1	7(3.66%)	3(3.16%)	4(4.17%)	
	MX	91(47.64%)	48(50.53%)	43(44.79%)	
Pathologic N	N0	95(49.74%)	44(46.32%)	51(53.12%)	0.0212
	N1	44(23.04%)	17(17.89%)	27(28.12%)	
	NX	52(27.23%)	34(35.79%)	18(18.75%)	
Pathologic T	T1	103(53.93%)	51(53.68%)	52(54.17%)	0.0154
	T2	54(28.27%)	20(21.05%)	34(35.42%)	
	T3	14(7.33%)	8(8.42%)	6(6.25%)	
	T4	9(4.71%)	6(6.32%)	3(3.12%)	
	TX	11(5.76%)	10(10.53%)	1(1.04%)	
NPC1 Expression	High	95(49.74%)	95(100%)	0(0%)	0
	Low	96(50.26%)	0(0%)	96(100%)	
Methylation	High	95(49.74%)	46(48.42%)	49(51.04%)	0.8279
	Low	96(50.26%)	49(51.58%)	47(48.96%)	

2.2 ONCOMINE Analysis

ONCOMINE database (https://www.oncomine.org/) is a prevalent data-mining platform, which is currently the world's largest cancer gene chip database. It contains the genome-wide-sequencing data of 86,733 cancerous tissues and healthy control tissue samples [17]. I collected four datasets *Pyeon Multi-cancer*, *Scotto Cervix*, *Zhai Cervix* and *Biewenga Cervix* to compare the mRNA level of NPC1 between cervical cancer and normal tissue. Threshold value: $p < 1E\text{-}4$, fold change > 1.4, gene rank: 10%, and data type: mRNA.

2.3 HPA database

The Human Protein Atlas (HPA) (http://www.proteinatlas.org/) a massive project based on protein examination, which contains more than 26,000 antibodies that target more than 17,000 human genes [18]. It clearly shows the expression of target genes at RNA and protein levels in different tissue and organs. I obtained six immunohistochemical results from the database on the expression of NPC1 protein in cervical

2.4 Gene Ontology (GO) Analysis

Gene Ontology (GO) analysis aims to provide a consistent description of gene functions in various databases [19]. GO analysis includes three major categories: biological process (BP), cellular component (CC) and molecular function (MF), which describe the possible molecular functions, subcellular structure and location of gene products, as well as the likely involved signaling pathways. GO analysis of differentially expressed genes can obtain more comprehensive gene function information. In this study, the R software Cluster Profiler package was used to annotate the function of the differential gene NPC1.

2.5 KEGG Enrichment Analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.kegg.jp/) is the most commonly used bioinformatics

analysis tool to explore signal pathways. It integrates a large number of high-throughput experimental data such as genome sequencing data into a network for analysis and research. KEGG mainly consists of three databases: the pathway database of interacting molecular networks (Pathway), the gene database of complete and partial sequencing (GENES) and the database of ligand chemical reactions (LIGAND). In this study, KEGG analysis was used to explore the NPC1 gene data set and biological function [20].

2.6 Connectivity Map (C-Map) Analysis

Connectivity map (C-Map) (https://portals.broadinstitute.org/C-Map/) is a biological application database, which can effectively identify candidate drugs for disease [21]. This database quickly matches drug molecules with high relevance to the disease through the use of gene expression data and deduce its related mechanism of action. NPC1 co-expressed genes were screened, positive and negative related genes were considered as up-regulated and down-regulated expression. Then, candidate drugs were screened by setting $p < 0.005$ and enrichment index < -0.8. Finally, the chemical structure formula and 2D and 3D structures of drugs were found in PubChem.

2.7 Statistical Analysis

R software (version 3.6.1) was used to conduct data analysis and processing in this study. The t-test was sued to compare the expression of NPC1 between cervical cancer and normal cervical tissue. The prognostic value of NPC1 was elevated through plotting Kaplan-Meier survival curves and ROC curves. In addition, Cox regression was utilized to prove whether up-regulation of NPC1 was independently associated with poor prognosis of cervical cancer. The P-values of all statistical analysis results was less than 0.05, which was considered as a significant result in this study.

3 RESULTS

3.1 NPC1 is Abnormally Highly Expressed in Cervical Cancer

I firstly compared the mRNA and protein level of NPC1 in cervical cancer tissue with normal tissue in ONCOMINE database and HPA database. Several datasets in ONCOMINE database reveal that the NPC1 mRNA level in cervical cancerous tissue was significantly increased than that in healthy tissue (Figure 1a-d). The *Pyeon Multi-cancer* dataset showed that the NPC1 mRNA expression in cervical cancer was 3.44 times higher than that in normal tissue (Figure 1a). Then, since proteins are the main executors of gene function, six clinical specimens were selected from the HPA database to analyze the level of NPC1 protein between normal and cervical cancer tissue. I selected three normal tissues and three cancer tissues using the same antibody CAB070132, and they were of similar basic characteristic. As clearly exhibited in Figure 1e-g, NPC1 protein was higher in cervical cancer group versus their expression levels in healthy control group. In summary, both mRNA and protein expression level of NPC1 was highly expressed in cervical cancer.

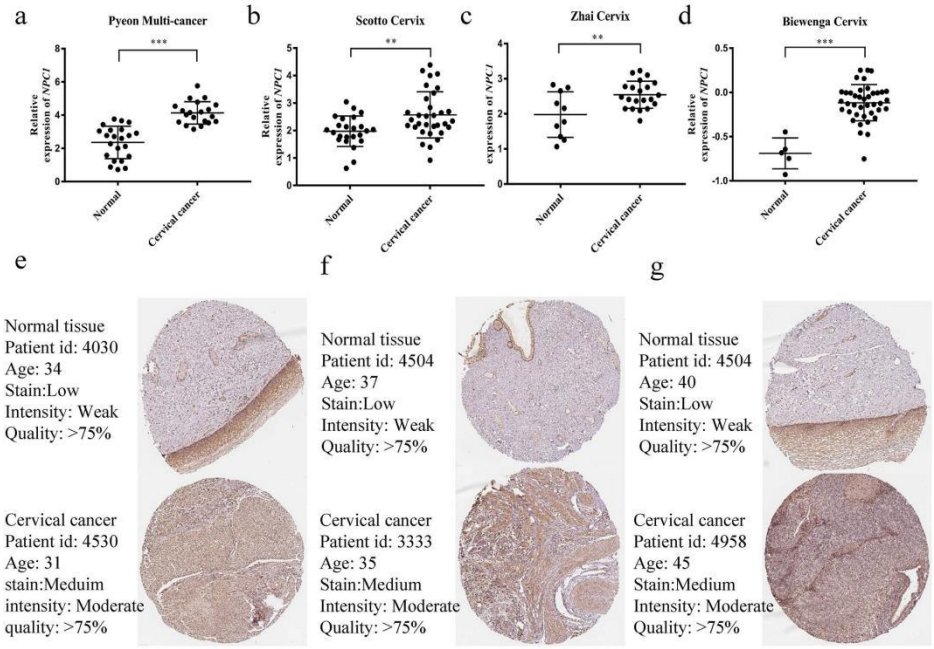


Figure 1 The Expression Level of NPC1 mRNA in Cervical Cancer is Significantly Higher than that in Normal Tissue: a-d: The mRNA Expression of NPC1 in Datasets of ONCOMINE; e-g: The Protein Expression Level of NPC1 Based on HPA Database

3.2 NPC1 High Expression Predicts Poor Prognosis in Cervical Cancer

Firstly, the patients were subdivided into high-NPC1 and low-NPC1 expression group according to the median of gene expression. Then, I performed the Kaplan-Meier survival analysis to underlying the association of survival probability and NPC1 expression level in cervical cancer patients. As shown in Figure 2a, the patients with high-NPC1 expression have a worse prognosis than those with low-NPC1 expression. Moreover, drawing ROC curve to evaluate the diagnostic value of NPC1 in cervical cancer. The area under the curve of 3-year and 5-year was respectively 0.683 and 0.762, indicating the overexpression of NPC1 had a relatively diagnostic value (Figure 2b).

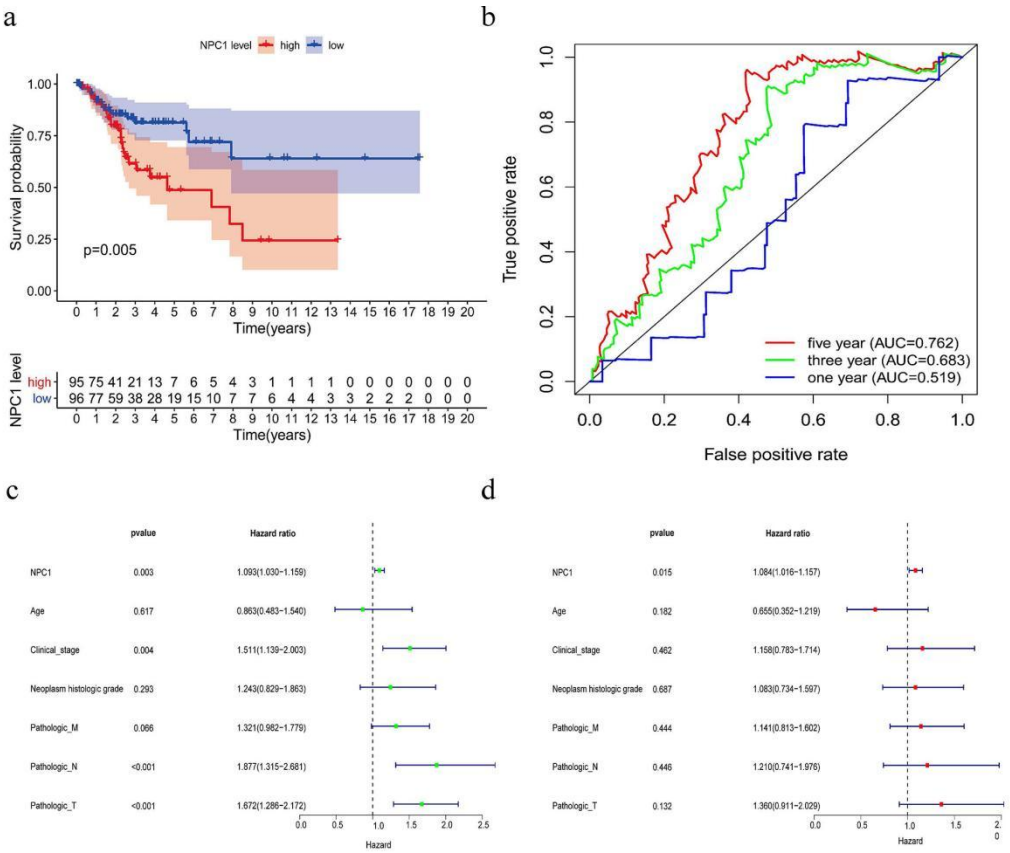


Figure 2 High Expression of NPC1 is Independently Related to the Poor Prognosis of Cervical Cancer: a. High expression of NPC1 indicates poor survival in cervical cancer; b. The ROC curve shows good diagnosis value of NPC1 expression in cervical cancer; c. Univariate regression of prognostic in patients with cervical cancer; d. Multivariate survival model of prognostic in patients with cervical cancer

3.3 NPC1 High Expression is an Independent Risk Factor in Cervical Cancer

Univariate and multivariate Cox regression analysis were conducted to find out factors that might arouse the poor prognosis of patients with cervical cancer. From the univariate Cox regression analysis results shown in Figure 2c, I found that high-NPC1 expression correlated significantly with poor survival (P=0.003, hazard ratio [HR]=1.093(95%CI [1.030-1.159])). Additionally, the clinical stage, pathologic N and pathologic T were risk factors for the poor prognosis as well. The subsequent multivariate Cox regression analysis was performed to eliminate the influence of confounding factors (Figure 2d). NPC1 expression remained independently associated with prognosis, with an HR of 1.084(95%CI [1.016-1.157], p=0.015). The above results emphasized that NPC1 was an independent risk factor for the prognosis of suffers with cervical cancer.

3.4 Functional Annotation and Pathway Enrichment Analysis of NPC1

GO and KEGG analyses were carried out to comprehensively understand the biofunction of NPC1. NPC1 might be of vital importance to gene expression and translation, because the BP results showed that NPC1 was involved in many biological processes such as nuclear transcribed mRNA catalytic process, translation initiation and viral gene expression and transcription (Figure 3a). CC analysis indicated that NPC1 contribute to the formation of cell-subject connection, focal adhesion and mitochondrial protein complex (Figure 3b). Also, MF revealed NPC1 was related to cell adhesion molecule binding, cadherin binding, ribonucleoprotein complex binding and other molecular functions (Figure 3c).
Subsequent KEGG analysis showed that NPC1 significantly enriched in the following metabolic pathway: cell cycle, FoxO, wnt and TGF-β signaling pathway (Figure 3d). These signaling pathway are closely related to the malignant

process of tumor and have drawn the attentions of cancer researchers worldwide.

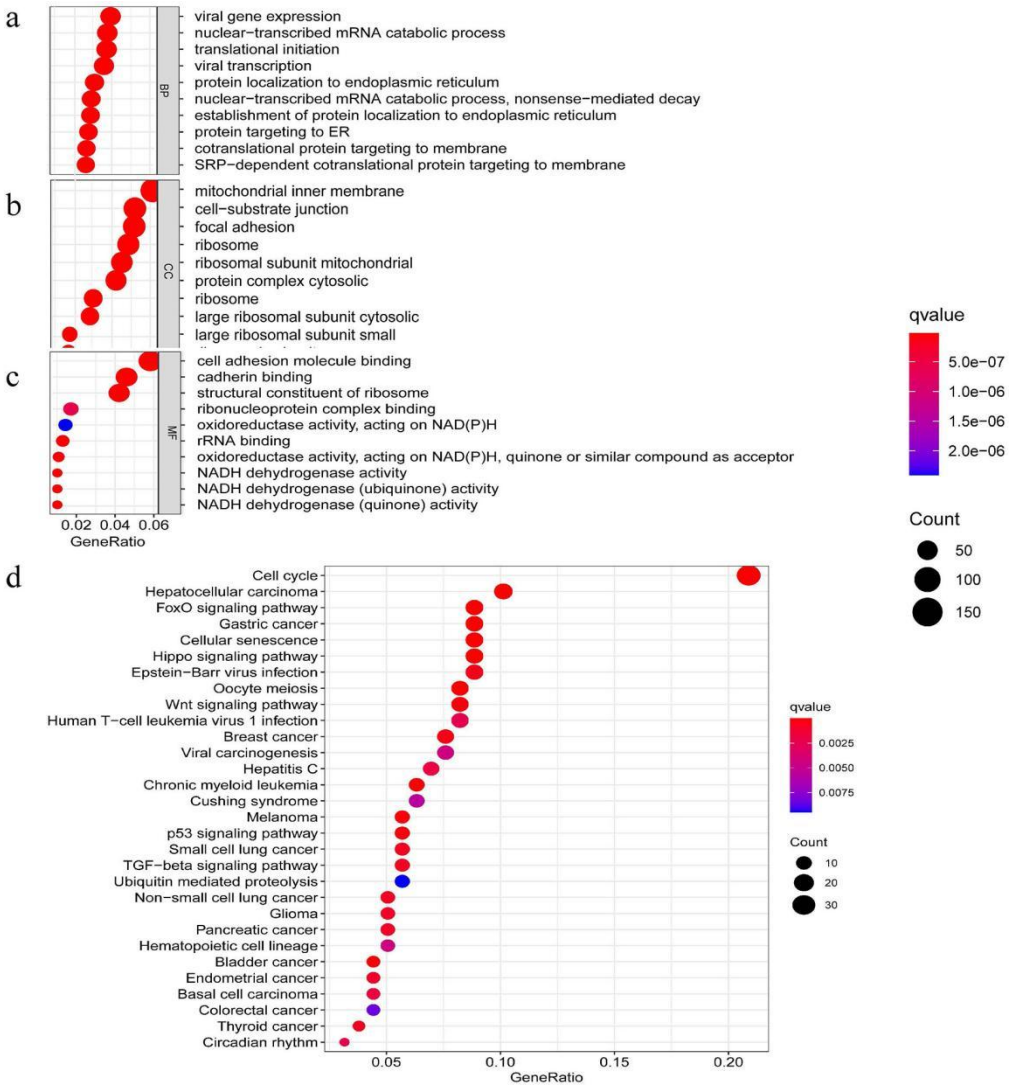


Figure 3 Gene Oncology Enrichment Analysis and KEGG Pathway Enrichment Analysis of NPC1: a. Biological Process (BP); b. Cellular Component (CC); c. Molecular Function (MF); d. The Most Related Signaling Pathway

3.5 Relationships of NPC1 with Immune in Cervical Cancer

The TISDB database was used to identify the relationship between NPC1 expression and the various types of lymphocytes, immunomodulator and chemokine in the tumor microenvironment. In cervical cancer, there was a significant positive correlation between the expression of NPC1 and the infiltration of immune cells, such as Tcm CD8 cell ($r = 0.317$, $p = 1.71e-8$), CD56bright ($r = 0.201$, $p = 0.000416$), neutrophils ($r=0.22$, $p = 0.000106$) and Tcm CD4 ($r = 0.281$, $p = 6.58e-7$) (Figure 4a). Notably, this positive correlation in cervical cancer was present between all kind of immune-inhibitors (TGFB1, CD274, PDCD1LG2 and TGFBR1) and NPC1 expression (Figure 4b), and negative correlation was present between all kinds of immune-stimulators (TNFRSF14, TNFRSF3C, CXCR4 and HHLA2) (Figure 4c). Finally, in cervical cancer, NPC1 expression was closely associated with chemokine of the tumor microenvironment (i.e., CXCL8, CDL22, CCL26 and CXCL6) (Figure 4d).

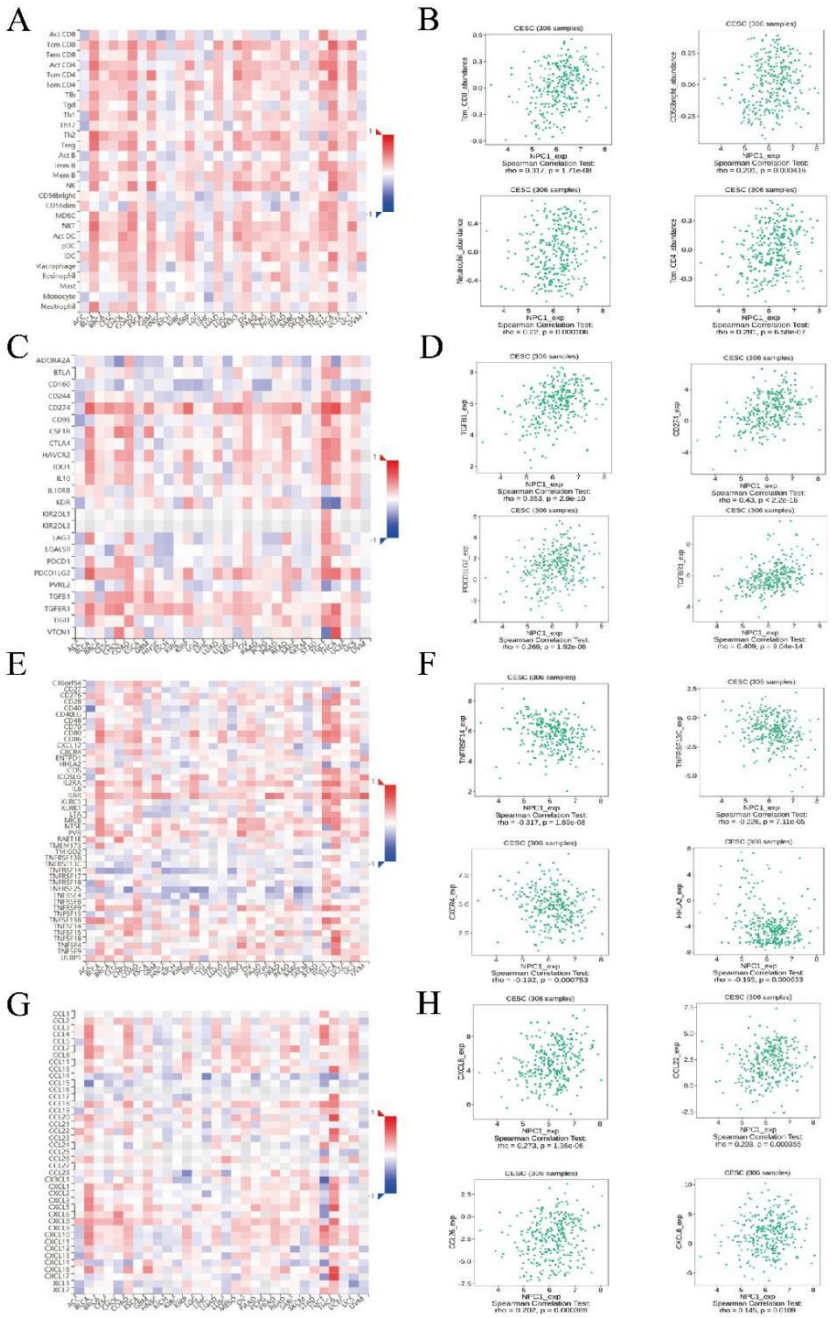
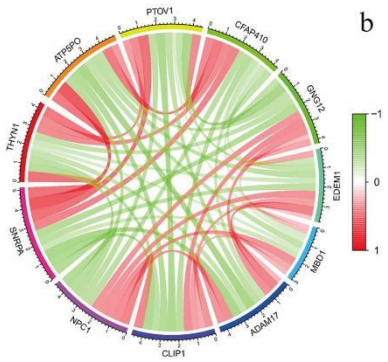


Figure 4 The Correlation of the Expression of NPC1 with Various Types of Lymphocytes, Immunomodulator and Chemokine In Cervical Cancer: a-b. Lymphocytes; c-d. Immune-Inhibitors; e-f. Immune-Stimulators; g-h. Chemokine

3.6 Co-Expression Analysis of NPC1 and Screening of Potential Drugs

Firstly, I used Pearson correlation analysis to explore the co-expression relationship of the NPC1 and other parameters. Based on the correlation coefficient, I obtained the ten genes that most positively or negatively associated with NPC1 expression in patients with cervical cancer. As Figure 5a and 5b showed that there were top five genes with the negative correlation with NPCI (SNRPA, THYN1, ATP5PO, PTOV1, CFAP410) and top-five genes with the positive correlation (CLIP1, ADAM17, MDB1, EDEM1, GNG12). Then, according to the gene principle of the C-Map database, I screened four small molecule compounds with potential therapeutic effects for cervical cancer, including mitoxantrone, harmol, fenoprofen and azacitidine. At last, I searched their chemical structure information in the platform of PubChem (Figure 5c-f).

a



b

gene	cor	pvalue
SNRPA	-0.477	6.54E-16
THYN1	-0.462	6.66E-15
ATP5PO	-0.446	7.33E-14
PTOV1	-0.439	1.89E-13
CFAP410	-0.436	2.90E-13
CLIP1	0.507	4.53E-18
ADAM17	0.47	2.20E-15
MBD1	0.46	9.75E-15
EDEM1	0.458	1.33E-14
GNG12	0.447	6.23E-14

c

Mitoxantrone
$C_{22}H_{28}N_4O_6$
PubChem CID:4212

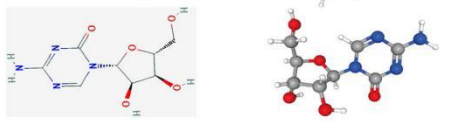
d

Harmol
$C_{12}H_{10}N_2O$
PubChem CID:68094

e

Fenoprofen
$C_{15}H_{14}O_3$
PubChem CID:3342

f



Azacitidine
$C_8H_{12}N_4O_5$
PubChem CID:9444

Figure 5 C-Map Analysis of NPC1 in Cervical Cancer: a-b. Top 10 Genes that are Positively and Negatively Correlated with NPC1 Expression (Co-Expression Networks and the Genes and Correlation Coefficient). c-f: The Small-Molecule Compounds that may Inhibit NPC1 Expression and Their 2D and 3D Structures

4 DISCUSSION

To my best knowledge, the biofunction of NPC1 has not been made known in cervical cancer, so this study aimed to explore the expression of NPC1 and its potential diagnostic and therapeutic value in cervical cancer. In this study, I firstly explored the transcription and translation levels of NPC1 in cervical cancer through ONCOMINE and HPA database. The expression results of NPC1 at the mRNA and protein levels verified each other and revealed that NPC1 was abnormally overexpressed in cervical cancer. A previous study showed that NPC1 was upregulated in hepatic cancer and enhanced the proliferation and migration of hepatic cancer cells [14]. Therefore, I speculated that NPC1 may be involved in the pathological process of cervical cancer as an oncogene.

To clarify the relationship between NPC1 expression and the prognosis of cervical cancer patients, I performed the Kaplan-Meier analysis. It is found that aberrant NPC1 overexpression implies a poor survive in cervical cancer. Subsequent ROC curves demonstrated that high NPC1 expression has prognostic value for poor 3-year and 5-year prognosis. However, it should be noticed that the overexpression of NPC1 has limited prognostic value for poor survival outcomes in the first year. Hence, NPC1 is more valuable for the prognosis in patients with long survival time. Combined the univariate and multivariate analysis results, increased NPC1 expression was an independent risk factor to the poor survival of patients. Also, its therapeutic and diagnostic value has already been verified in other cancer types. For instance, NPC1 was considered as a potential biomarker of the ability of human breast carcinoma cancer cells to colonize and establish metastasis [22]. A prognostic risk model composed by NPC1 can accurately forecast the prognosis of glioma patients [23]. Based on above results, NPC1 represented remarkably potential to be a promising biomarker for cervical cancer. However, how NPC1 participates in the pathological progression of cervical cancer is limited to understand.

To explore the specific mechanism of NPC1 as an oncogene in the progression of malignance, I investigated the cell signaling pathway that NPC1 may involve in cervical cancer through KEGG analysis. The results demonstrated that NPC1 was involved in the cell cycle, FoxO, wnt and TGF-β signaling pathway. The significance of these pathways has been proved in a variety of cancer types including cervical cancer. For example, the wnt signaling pathway plays a crucial regulatory function in the malignant progression of cervical cell, which is upregulated by the SALL4, increasing the tumorigenicity of cervical cancer cell [24]. Promoting the FoxO signaling pathway could significantly suppress cell proliferation, cell invasion and facilitate cell apoptosis in cancer cell [25]. Silenced HOXC6 gene caused by TGF-β signaling pathway can inhibit the epithelial-mesenchymal transition (EMT) of cervical cancer [26]. Though KEGG

analysis elucidated the function of NPC1 in cervical cancer indirectly, the results can be considered credible as they are based on comparisons between NPC1 and tens of thousands of genes to match the most likely pathways. Many researchers have used KEGG analysis method to obtained valuable results. For instance, Cai et al and Zhao et al identified two impressive prognostic prediction biomarkers for cervical cancer through this method [27-28].

Pearson correlation analysis was conducted to explore the expression correlation between NPC1 and other genes. Gene co-expression results demonstrated that NPC1 is positively correlated with other oncogenes, such as GNG12, MBD1 and ADAM17. Their carcinogenic function has been already revealed in previous studies. For example, high GNG12 enhances the growth of pancreatic cancer cell and indicates a poor prognosis in patients with pancreatic cancer [29]. The aggressive progression and poor prognosis are significantly related to ADAM17 in uterine cervical carcinoma [30]. What is more, overexpression MBD1 promoted cell proliferation and metastasis in cervical cancer and gallbladder cancer [31-32]. My results are supported by predecessors, and it also shows that my results have a certain credibility.

Given the crucial character of NPC1 in the oncogenesis and progression in cervical cancer, NPC1 might be an attractive target for cancer therapy. Based on the genome-wide transcriptional expression data from cultured human cells treated with small bioactive molecules in C-Map database, four small-molecule compounds, mitoxantrone, harmol, fenoprofen and azacitidine [33], were identified with inhibitory effect on NPC1 expression. Although these four drugs have not been used to treat cervical cancer, their antitumor activity is increasingly mentioned in other cancers' management. For example, mitoxantrone, a novel antineoplastic agent that can kill cancer cells of any cell cycle, has been applied in the treatment of malignant lymphoma, ovarian cancer, breast cancer and bladder cancer [34-35]. Harmol induced the autophagy and apoptosis in U251MG human glioma cells by suppressing the expression of survivin [36]. Above all, the results of C-Map analysis enriched the application of the above-mentioned drugs and offered new options for the treatment of cervical cancer.

In my work, I revealed the close relationship between NPC1 and cervical cancer according to a wide range of analyses using data from multiple public databases. However, there were some unavoidable limitations exist in my analysis. First, the number of healthy samples obtained from the TCGA database was small compared to the tumor sample size, the unbalance in quantity may cause statistical errors. Hence, I explored the NPC1 expression level in several datasets in the ONCOMINE database to avoid statistical bias. Second, clinical information incomplete and inconsistency treatment were unignored short-coming of public databases because the experiments were conducted in different laboratories. However, the incomparable strengths of public databases are that massive sample information can be obtained in a short time, and the combined results are highly credible and applicable. Finally, my retrospective analysis indirectly revealed the prognostic value and likely mechanism by which NPC1 overexpression causes poor survival of patients with cervical cancer. In the meantime, the advantages of this study cannot be ignored: this article revealed NPC1 is a risk factor with high prognostic value for cervical cancer patients. What's more, I initially explored the oncogenic function of NPC1 and its potential therapeutic value. All in all, the present results are inspiring and striking in the field of identifying potential prognostic biomarker for cervical cancer.

In conclusion, I observed an increase in NPC1 expression levels in cervical cancer associated with poor prognosis, which could be served as an independent prognostic factor. In addition, NPC1 may participate in the pathological process of cervical cancer through the cell cycle and the wnt pathway. The results supply a promising candidate target for the diagnosis and individualized treatment of cervical cancer.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

REFERENCE

[1] Arbyn M, Iiderpass E, Bruni L, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob Health, 2020, 8(2): e191-e203.

[2] Smith R, Andrews K, Brooks D, et al. Cancer screening in the United States, 2019: A review of current American Cancer Society guidelines and current issues in cancer screening. CA: A Cancer Journal for Clinicians, 2019, 69(3): 184-210.

[3] von Karsa L, Arbyn M, De Vuyst H, et al. European guidelines for quality assurance in cervical cancer screening. Summary of the supplements on HPV screening and vaccination. Papillomavirus Research, 2015, 1: 22-31.

[4] Cohen A, Roane B, Leath C. Novel Therapeutics for Recurrent Cervical Cancer: Moving Towards Personalized Therapy. Drugs, 2020, 80(3): 217-227.

[5] Li H, Wu X, Cheng X. Advances in diagnosis and treatment of metastatic cervical cancer. Journal of Gynecologic Oncology, 2016, 27(4): e43.

[6] Du H, Chen Y. Competing endogenous RNA networks in cervical cancer: function, mechanism and perspective. Journal of Drug Targeting, 2019, 27(7): 709-723.

[7] Chaichian S, Shafabakhsh R, Mirhashemi S, et al. Circular RNAs: A novel biomarker for cervical cancer. The Journal of Cellular Physiology, 2020, 235(2): 718-724.

[8] Kim B, Cho H, Ylaya K, et al. Bcl-2-like Protein 11 (BIM) Expression Is Associated with Favorable Prognosis for Patients with Cervical Cancer. Anticancer Research, 2017, 37(9): 4873-4879.

[9] Stiasny A, Kuhn C, Mayr D, et al. Immunohistochemical Evaluation of E6/E7 HPV Oncoproteins Staining in Cervical Cancer. Anticancer Research, 2016, 36(6): 3195-3198.
[10] Fan S, Zhao S, Gao X, et al. Circular RNA circGSE1 Promotes Cervical Cancer Progression Through miR-138-5p/Vimentin. OncoTargets and Therapy, 2020, 13: 13371-13386.
[11] Rossi P, Carozzi F, Ronco G, et al. p16/ki67 and E6/E7 mRNA accuracy and prognostic value in triaging HPV DNA-positive women. Journal of the National Cancer Institute, 2020, 114(2): 324.
[12] Wang R, Pan W, Jin L, et al. Human papillomavirus vaccine against cervical cancer: Opportunity and challenge. Cancer Letters, 2020, 471: 88-102.
[13] Yu X, Jiang N, Yao P, et al. NPC1, intracellular cholesterol trafficking and atherosclerosis. Clinical Chemistry and Acta, 2014, 429: 69-75.
[14] Du X, Zhang Y, Jo S, et al. Akt activation increases cellular cholesterol by promoting the proteasomal degradation of Niemann-Pick C1. Biochemical Journal, 2015, 471(2): 243-253.
[15] Singh V, Singh L, Vasudevan M, et al. Esophageal Cancer Epigenomics and Integrome Analysis of Genome-Wide Methylation and Expression in High Risk Northeast Indian Population. OMICS, 2015, 19(11): 688-699.
[16] Iinstein J, Collisson E, Mills G, et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nature Genetics, 2013, 45(10): 1113-1120.
[17] Rhodes D, Yu J, Shanker K, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia, 2004, 6(1): 1-6.
[18] Thul P, Lindskog. The human protein atlas: A spatial map of the human proteome. Protein Science, 2018, 27(1): 233-244.
[19] Denny P, Feuermann M, Hill D, et al. Exploring autophagy with Gene Ontology. Autophagy, 2018, 14(3): 419-436.
[20] Xing Z, Chu C, Chen L, et al. The use of Gene Ontology terms and KEGG pathways for analysis and prediction of oncogenes. Biochimica et Biophysica Acta, 2016, 1860: 2725-2734.
[21] Musa A, Ghoraie L, Zhang S, et al. A review of connectivity map and computational approaches in pharmacogenomics. Briefings in Bioinformatics, 2018, 19(3): 506-523.
[22] Lund R, Leth-Larsen R, Caterino T, et al. NADH-Cytochrome b5 Reductase 3 Promotes Colonization and Metastasis Formation and Is a Prognostic Marker of Disease-Free and Overall Survival in Estrogen Receptor-Negative Breast Cancer. Molecular & Cellular Proteomics, 2015, 14(11): 2988-2999.
[23] Xu Y, Li R, Li X, et al. An Autophagy-Related Gene Signature Associated With Clinical Prognosis and Immune Microenvironment in Gliomas. Frontiers in Oncology, 2020, 10: 571189.
[24] Bahrami A, Hasanzadeh M, ShahidSales S, et al. Clinical Significance and Prognosis Value of Wnt Signaling Pathway in Cervical Cancer. Journal of Cellular Biochemistry, 2017, 118(10): 3028-3033.
[25] Shan Z, Li Y, Yu S, et al. CTCF regulates the FoxO signaling pathway to affect the progression of prostate cancer. Journal of Cellular and Molecular Medicine, 2019, 23(5): 3130-3139.
[26] Zhang F, Ren C, Liu L, et al. HOXC6 gene silencing inhibits epithelial-mesenchymal transition and cell viability through the TGF-β/smad signaling pathway in cervical carcinoma cells. Cancer Cell International, 2018, 18: 204.
[27] Cai S, Yu X, Gu Z, et al. A 10-gene prognostic methylation signature for stage I-III cervical cancer. Archives of Gynecology and Obstetrics, 2020, 301(5): 1275-1287.
[28] Zhao S, Yu C. MMP1Identification of as a Potential Prognostic Biomarker and Correlating with Immune Infiltrates in Cervical Squamous Cell Carcinoma. 2020, 39(2): 255-272.
[29] Li J, Jin C, Zou C, et al. GNG12 regulates PD-L1 expression by activating NF-κB signaling in pancreatic ductal adenocarcinoma. FEBS Open Bio, 2020, 10(2): 278-287.
[30] Xu Q, Ying M, Chen G, et al. ADAM17 is associated with EMMPRIN and predicts poor prognosis in patients with uterine cervical carcinoma. Tumor Biology, 2014, 35(8): 7575-7586.
[31] Liu D, Huang K, Wang T, et al. NR2F2-AS1 accelerates cell proliferation through regulating miR-4429/MBD1 axis in cervical cancer. Bioscience Reports, 2020, 40(6).
[32] Insheng L, Bo Z, Qiangsheng H, et al. MBD1 promotes the malignant behavior of gallbladder cancer cells and induces chemotherapeutic resistance to gemcitabine. Cancer Cell International, 2019, 19: 232.
[33] Li Y, Hu W, Shen D, et al. Azacitidine enhances sensitivity of platinum-resistant ovarian cancer cells to carboplatin through induction of apoptosis. American Journal of Obstetrics and Gynecology, 2009, 200(2): 177.e1-9.
[34] Lin R, Steinmetz N. Tobacco mosaic virus delivery of mitoxantrone for cancer therapy. Nanoscale, 2018, 10(34): 16307-16313.
[35] Faulds D, Balfmy J, Chrisp P, et al. Mitoxantrone. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in the chemotherapy of cancer. Drugs, 1991, 41(3): 400-449.
[36] Abe A, Kokuba H. Harmol induces autophagy and subsequent apoptosis in U251MG human glioma cells through the downregulation of survivin. Oncology Reports, 2013, 29(4): 1333-1342.