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ORIGINAL RESEARCH

NDC1 is a Prognostic Biomarker and Associated with Immune Infiltrates in Colon Cancer

Meng Liu[1,*]
Rui Yuan[2,*]
Shifei Liu[3]
Yonggan Xue[2]
Xuning Wang[4]

[1]Department of Integrative Oncology, China-Japan Friendship Hospital, Beijing, 100029, People's Republic of China; [2]Department of Critical Care Medicine, Chinese PLA General Hospital, Beijing, 100853, People's Republic of China; [3]Department of Pathology, Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing, 100026, People's Republic of China; [4]Department of General Surgery, The Air Force Hospital of Northern Theater PLA, Shenyang, 110000, People's Republic of China

*These authors contributed equally to this work

Background: Colon cancer is one of the most lethal cancers in the world. NDC1 is a crucial membrane-integral nucleoporin of nuclear pore complexes. The clinical significance of NDC1 in colon cancer has not been demonstrated to date. Therefore, we determined to evaluate the association between NDC1 and colon cancer using the open-access database.

Methods: The TCGA data of colon cancer were extracted to determine the relationship between NDC1 and the clinical characterization. We assessed the predictive role of NDC1 expression in the survival of patients with colon cancer. Univariate and multivariate Cox proportional hazard models were applied to analyze the association between the clinical factors and prognosis. The TIMER database was used to describe the association between immune cell infiltration and specific gene expression in the colon cancer context. Gene set enrichment analysis (GSEA) was performed based on the TCGA dataset.

Results: A total of 445 colon cancer patients with complete clinical information were included. NDC1 expression was significantly up-regulated in colon cancer tissues compared to adjacent normal tissues. Univariate and multivariate Cox regression analyses showed that NDC1 was an independent prognostic factor. Patients with a higher level of NDC1 expression tend to survive longer compared to those with a lower level of NDC1 expression. The level of the NDC1 expression is significantly associated with TNM stages. Furthermore, we constructed a nomogram to predict the prognosis by using NDC1 as a factor. The expression of NDC1 was significantly associated with infiltration of B cell, CD8+T cells, macrophages, neutrophils, and dendritic cells in colon cancer lesions. Additionally, NDC1 was predominantly enriched in KRAS-related signaling pathways by GSEA.

Conclusion: NDC1 can serve as a prognostic biomarker, which is negatively correlated with aggressiveness and positively associated with immune infiltrates of colon cancer.

Keywords: NDC1, colon cancer, immune infiltrates, prognostic biomarker

Introduction

Colon cancer is one of the most common cancers and the leading cause of cancer death around the world, ranking fourth for incidence and third for mortality, which is becoming a tough barrier to increase the life expectancy of the patients across countries in the 21st century.[1,2] Benefiting from the improved surgical techniques and devices, diagnostic and monitoring strategies and chemical and immunological therapy, the five-year survival rate of the disease had been increased by 0.9%, from 63.7% to 64.6% between 2001 and 2009,[3] especially for younger ages (20 ~ 54 years).[4,5] Many factors have been listed as crucial contributors, such as heavy smoking and alcohol abuse to inducing colon cancer.[6] However, the fundamental mechanism dissecting the evolution of colon cancer remains elusive.[7] Thus, deeply

Correspondence: Xuning Wang
Department of General Surgery, The Air Force Hospital of Northern Theater PLA, Shenyang, 110000, People's Republic of China
Email 15210511316@126.com

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studying genomic characteristics of colon cancer is expected to light the tunnel of basically understanding the onset of the disease.

NDC1 is a transmembrane nucleoporin that plays a crucial role in nuclear pore complexes assembly and nucleocytoplasmic transport. The lack of NDC1 disrupts the nuclear assembly by impairing the localization of FG (Phe-Gly) repeat-containing nucleoporins. NDC1 interacts with ALADIN which is responsible for Allgrove syndrome, suggesting NDC1 is also involved in Allgrove syndrome.[8] Researchers found the NDC1 interacted with SEPT12 which is required for mammalian spermiogenesis.[9] All those studies demonstrate that NDC1 serve a important role in the biological process. Despite the involvement of NDC1 in many important biological processes, the role of NDC1 in colon cancer remains unclear. Here we utilized bioinformatic approaches to identify the role of NDC1 in predicting the prognosis of colon cancer and analyze the relationship with the clinical factors with NDC1.

Materials and Methods

Data Preparation

Datasets collected from colon cancer in The Cancer Genome Atlas (TCGA), including gene expression profiles and clinical information, were downloaded via XENA (http://xena.ucsc.edu/).[10]

Relationship Between NDC1 Expression with Prognosis and Clinical Factors

Univariate and multivariate Cox proportional regression was employed to analyze the relationship between the NDC1 and prognosis. Based on the median, patients with colon cancer were divided into two groups. Survival analysis was performed using the Kaplan–Meier method. The different expression of NDC1 between groups was compared by ANOVA or Wilcoxon test.

Association Between NDC1 Expression and Immune Infiltrates

TIMER (https://cistrome.shinyapps.io/timer/),[11] a web tool to analyze the immune infiltrates across TCGA samples, was used to explore the correlation between NDC1 expression and immune infiltrates. The tumor immune infiltrates, including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells, were analyzed via gene modules.[12]

Gene Set Enrichment Analysis

Gene set enrichment analysis is a computational method that can be performed to assess the statistical significance of a pre-defined gene and its concordant expression differences between two biological states.[13]

The nominal p-value and normalized enrichment score were set to allocate the enriched pathways in individual

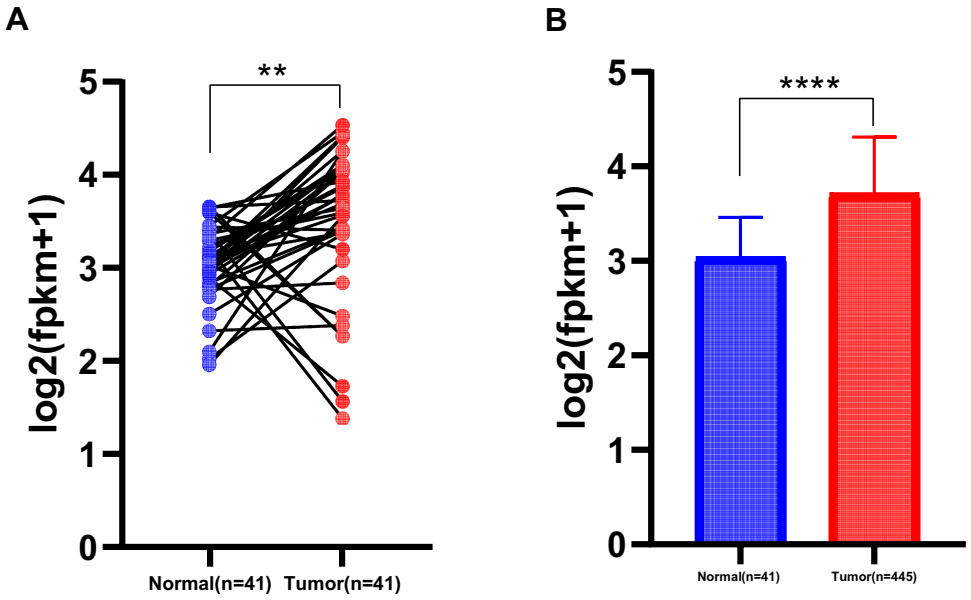

Figure 1 NDC1 was upregulated in colon cancer tissue. (**A**) NDC1 was upregulated in tumor compared with paired samples. (**B**) NDC1 was upregulated in colon cancer cohort. (**p < 0.01, ****p < 0.001).

phenotype.[14] Gene sets with a discovery rate (FDR) <0.05 were considered to be statistically significant.

Statistical Analysis

The statistical analysis was all conducted by R-3.6.0. Survival curves are plotted using the R package.[15] P-value ≤0.05 was considered as the cut-off criterion.

Results

The Expression of NDC1 and Its Correlation with Survival in Colon Cancer

A total of 445 samples including 214 females and 237 men and another 41 normal samples as control were included in the study (Supplementary Table).

Figure 1A shows that NDC1 is highly expressed in cancer tissues compared with matched adjacent tissues. Figure 1B shows NDC1 was upregulated in tumor samples in colon cancer cohort. Univariate and multivariate Cox proportional hazard regression models showed that NDC1 was an independent prognosis factor for colon cancer (Table 1). Additionally, lymph node was also the independent prognosis factor in our analysis (Table 1).

Furthermore, we explored the relationship of NDC1 with clinical factors. We found that the expression of NDC1 in patients with stage III and IV was higher than in patients with stage I and II (Figure 2A). Similarity trend was shown in T(tumor) stage (Figure 2B), N(lymph) stage (Figure 2C) and M(metastasis) stage (Figure 2D).

Table 1 Results of Univariate and Multivariate Cox Regression Analysis

Characteristics	Univariate Cox Regression Analysis				Multivariate COX Regression Analysis			
	HR	95% CI		P value	HR	95% CI		P value
		Low	High			Low	High	
Age	1.03	1.01	1.05	0.00	1.04	1.02	1.06	0.00
Gender								
Female	Reference				Reference			
Male	1.09	0.73	1.63	0.66	0.89	0.59	1.35	0.59
Stage								
I	Reference				Reference			
II	3.85	2.50	5.94	0.00	1.46	0.14	14.99	0.75
III	1.49	0.89	2.49	0.13	8.29	0.69	99.01	0.09
IV	3.75	2.37	5.93	0.00	15.43	1.30	182.57	0.03
Tumor								
T1	Reference				Reference			
T2	2.01	0.77	5.21	0.15	0.51	0.05	4.91	0.56
T3	3.39	1.32	8.73	0.01	0.79	0.04	15.69	0.88
T4	8.65	3.36	22.27	0.00	1.77	0.09	36.04	0.71
Tis	0.35	0.06	1.92	0.23	0.00	0.00	Inf	1.00
Lymph node								
N0	Reference				Reference			
N1	1.21	0.30	4.94	0.79	0.26	0.09	0.74	0.01
N2	3.27	0.76	14.04	0.11	0.52	0.19	1.42	0.20
Metastasis								
M0	Reference				Reference			
M1	0.00	0.00	Inf	0.99	NA	NA	NA	NA
NDC1								
Low	Reference				Reference			
High	0.55	0.37	0.84	0.01	0.61	0.40	0.93	0.02

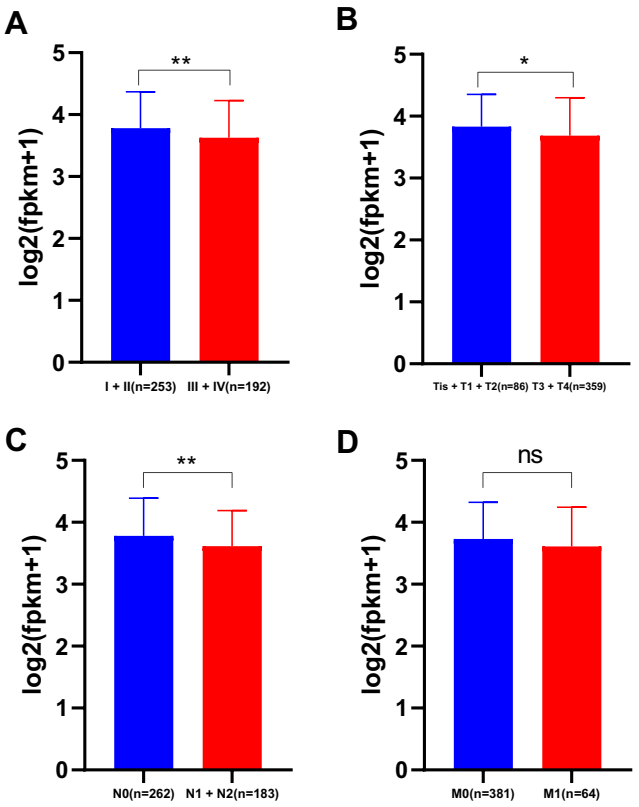

Figure 2 With tumor stage increasing, the expression of NDC1 was decreased. (**A**) The expression of NDC1 in stage III and IV was higher than stage I and II. (**B**) NDC1 was decreased in T3 and T4 stage compared with Tis, T1 and T2. (**C**) NDC1 was down-regulated in N1 and N2 compared with N0. (**D**) The expression of NDC1 in M stage also showed similar trend. (*p < 0.05, **p < 0.01).
Abbreviation: ns, not significance.

Nomogram with NDC1

We divided patients with colon cancer into 2 groups based on the expression of NDC1. Patients with higher expression of NDC1 tend to live longer than those with a lower level of NDC1 expression (Figure 3A).

With the results mentioned above, NDC1 served as a predictive biomarker. A nomogram was plotted to predict the overall survival of colon cancer (Figure 3B). C-index was up to 0.77.

Association Between NDC1 Expression and Tumor-Infiltrating Immune Cells

Previous studies have demonstrated that tumor-infiltrating lymphocytes serve as independent predictors for survival in cancer patients and favorable marker for immune therapy.[16,17] Thus, we attempted to find out whether NDC1 expression was associated with immune infiltration in colon cancer. The expression of NDC1 was significantly associated with B cell (r = 0.246, p = 5.38e-7), CD8+ T cell (r = 0.299, p = 7.38e-10), CD4+ T cell (r = 0.16, p = 1.33e-3), Macrophage (r = 0.406, p = 2.40e-17), Neutrophil (r = 0.406, p = 2.40e-17) and Dendritic cell (r = 0.34, p = 2.34e-12) (Figure 4).

Gene Sets Enrichment of the High Expression Phenotype

All the samples involved in the present study were divided into two groups (high expressed and low expressed group) based on the expression of NDC1. The top enriched pathways are listed in Figure 5. It is shown that NDC1 is preferably enriched in KRAS, LEF1, PTEN, and VEGF-related pathways.

Discussion

NDC1 is crucial for membrane-integral nucleoporin in metazoan nuclear pore complexes and is involved in the transportation biological process. Human NDC1 is located

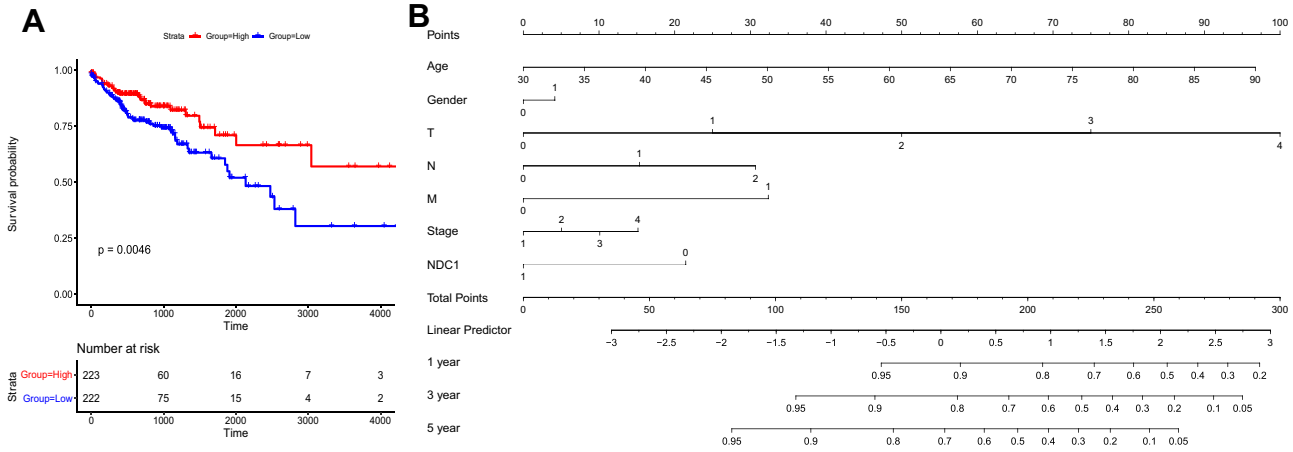

Figure 3 NDC1 was prognosis biomarker of survival. (**A**) Colon cancer patients were divided into two groups. Patients with higher expressed NDC1 had better survival. (**B**) The nomogram was showed for colon cancer with NDC1 as clinical factor.

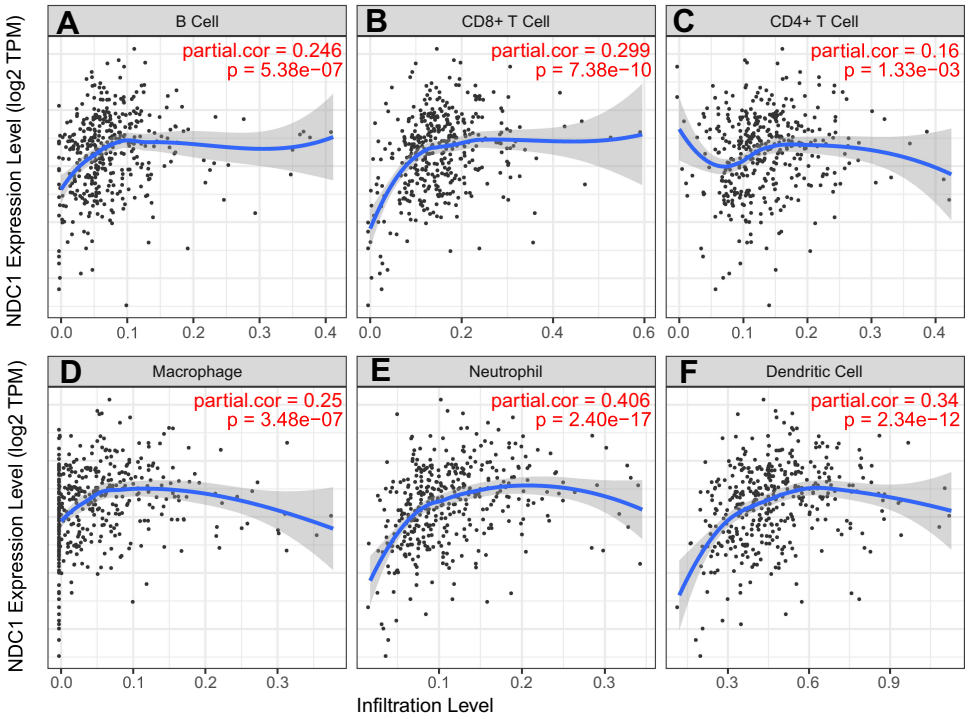

Figure 4 The expression level of NDC1 was correlated with immune cells, B cell (r = 0.246, p = 5.38e-7), CD8+ T cell (r = 0.299, p = 7.38e-10), CD4+ T cell (r = 0.16, p = 1.33e-3), Macrophage (r = 0.406, p = 2.40e-17), Neutrophil (r = 0.406, p = 2.40e-17) and Dendritic cell (r = 0.34, p = 2.34e-12) (**A**–**F**).

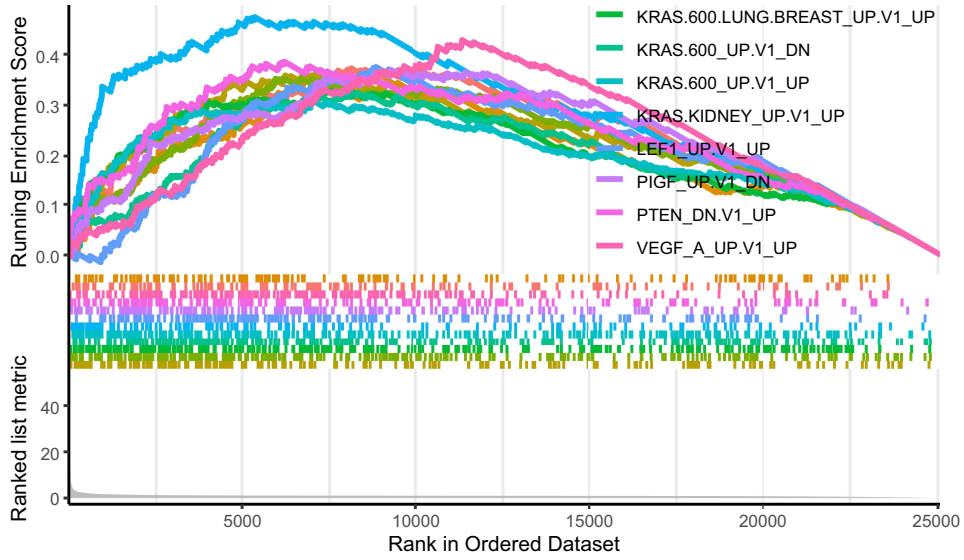

Figure 5 GSEA analysis revealed NDC1 was involved many import pathways. Top 8 pathways were graphed.

at the nuclear pore wall and consists of six transmembrane segments.[18] Loss of NDC1 was not always ultimately lethal for cells, but it could still cause severe nuclear pore complex defects, which have a crucial influence on the exchange of metabolites and macromolecules across the nuclear compartment and cytoplasm.[19]

Previous studies on NDC1 mainly focus on the role of membrane nucleoporin. However, few studies have explored the clinical significance of NDC1 in colon cancer. Therefore, we conduct this research to explore whether NDC1 is involved in the progress of colon cancer.

We found that NDC1 was significantly upregulated in tumor samples when compared with normal tissue. Moreover, our analyses revealed that the higher the tumor stage, the lower level of NDC1 expression was observed. Additionally, patients with up-regulated NDC1expression have a better chance of surviving longer than those with a lower level of NDC1 expression. Univariate and multivariate Cox regression analyses indicated that NDC1 was an independent prognosis factor. All those results indicated that NDC1 may serve as a tumor suppressor gene in the progress of colon cancer evolving. However, some studies have demonstrated that NDC1 is upregulated in lung cancer samples, and elevated expression of NDC1 is associated with poor prognosis for the disease.[20,21]

The contradictory findings of NDC1 in different contexts of cancer, such as colon cancer and lung cancer, indicate that the role of NDC1 in the development of malignant events is organ-specific. Further steps forwards to decipher the underlying mechanism of the gene in the progress of malignant carcinoma are urgently required to handle the lethal disease.

With NDC1 severing as a clinical factor, a nomogram was plot which C-index up to 0.77. Additionally, to explore the relationship between the NDC1 and the immune infiltrates of cancer, we used the TIMER dataset and found that NDC1 was statistically associated with infiltration of B cell (r = 0.246, p = 5.38e-7), CD8+ T cell (r = 0.299, p = 7.38e-10), CD4+ T cell (r = 0.16, p = 1.33e-3), Macrophage (r = 0.406, p = 2.40e-17), Neutrophil (r = 0.406, p = 2.40e-17) and Dendritic cell (r = 0.34, p = 2.34e-12). These findings suggest the presence of NDC1 is correlated with the immune microenvironment of colon cancer. Our results indicate that NDC1 may play a crucial role in the immune-related biological process in the progression of colon cancer.

Further analysis suggested that KRAS, LEF1, PIGF, PTEN, and VEGF-related pathways, which were crucial for the development of colon cancer, were enriched due to the expression of NCD1.

Conclusion

NDC1 can be used as a clinical biomarker for predicting the prognosis of colon cancer. NDC1 was associated with tumor stages. Additionally, the level of NDC1 expression is also associated with immune infiltrates in the lesion. All these findings indicate that NDC1 may play an important role in the progress of colon cancer evolution.

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Disclosure

Meng Liu and Rui Yuan are co-first authors of this study. The authors report no conflicts of interest in this work.

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