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NIFK as a potential prognostic biomarker in colorectal cancer correlating with immune infiltrates

Lin Tan, MD[a,b], Yuqi He, BSc[b], Linhui Wu, BSc[b], Shiquan Liu, PhD[a], Peng Peng, PhD[a], Jiean Huang, PhD[a,*]

Abstract

Background: Immune-related initiation, progress, metastasis and sensitivity to treatment associated with poor prognosis of patients with colorectal cancer (CRC). The role of Nucleolar protein interacting with the FHA domain of MKI67 (NIFK) in CRC remained to be investigated. We explore whether NIFK correlates with tumor immune infiltration and plays an important role in CRC patient prognosis.

Methods: The data of samples involved in our study was obtained from TCGA and GEO and samples for protein expression detection and clinical information analysis were obtained from our hospital. NIFK expression, association with patient prognosis, correlation with infiltration of immune cells and its correlated genes involved in signaling pathways were analyzed using bioinformatics method along with experimental validation and clinical correlation analysis.

Results: Results indicated that the expression of NIFK in tumor tissues was significantly increased compared with normal samples. colon and rectal cancer patients with high NIFK expression have poor survival compared with those with low NIFK expression. Results of cell experiments indicated that NIFK is positively correlated with cell proliferation and migration in CRC. NIFK negatively correlated with T cell CD8+, Tregs, Neutrophil and macrophage significantly. DARS and NKRF were positively correlated with NIFK and DARS correlated with CD8 + T cell, CD4 + T cell, macrophage and Neutrophil, NKRF correlated with CD8 + T cell, CD4 + T cell and macrophage in colon and rectal cancer. NIFK along with its correlated genes as DARS and NKRF were involved in Wnt, PI3K-Akt, NF-κB signaling and Intestinal immune network for IgA production.

Conclusions: Our results suggested that NIFK might be a biomarker associated with poor prognosis of CRC patients, and it would be a potential target for CRC therapy.

Abbreviations: CRC = colorectal cancer, NETs = neutrophil extracellular traps.

Keywords: colorectal cancer, immune infiltrates, NIFK, prognostic biomarker

1. Introduction

Colorectal cancer (CRC) was one of the most common cancers with high incidence and mortality.[1] Drug resistance, metastasis and recurrence remain leading causes related to survival of patients with CRC.[2–4] Therefore, it needs to explore markers associated with tumor progression to improve prognosis of patients with CRC.

The stromal immune landscape and tumor microenvironment are important in cancer development.[5] Chemotherapy efficacy may be predicted by biomarkers of in the tumor immune microenvironment of CRC.[5] According to He et al, tumor immune cell infiltration-related complement component 3 (C3) gene expression associated resistance of FOLFOX chemotherapy involved in 5-fluorouracil and oxaliplatin.[6] Bothe immune landscape of and chemotherapy response in CRC which due to predict prognosis of patients may be related to sialylation-related long noncoding RNAs characterization.[7] In another study, Fucoidan was considered as an immunostimulator to promote M1 macrophage differentiation to enhance chemotherapeutic sensitivity of patients with colon cancer to capecitabine.[8]

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The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

This study was approved by the ethics committee of the he Affiliated Zhuzhou Hospital Xiangya Medical College CSU (No.: 2018K0327), and all patients signed an informed consent form.

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The tumor microenvironment including various immune cells serve to tumor initiation and progression of CRC.[9] Multiple immune contexture contributed to survival of cancer patients. Metastasis and recurrence play a vital role in CRC progression and associated with immune parameters in patients.[10] As showed in many studies,[11–13] epithelial-mesenchymal transition played a critical role in the tumor metastasis and tumor microenvironment including immune landscape contributed epithelial-mesenchymal transition development.[14] Immune related IL-6/STAT3 pathway with its downstream genes could drive tumor invasion and metastasis along with drug resistance.[15] Inhibitors of immune checkpoint could benefit metastatic CRC with a mismatch repair-deficiency/microsatellite instability-high phenotype.[16]

Nucleolar protein interacting with the FHA domain of MKI67(NIFK) could bind RNA and plays an important role in mitosis and cell cycle progression.[17] Neutrophil extracellular traps (NETs)-related NIFK was significantly upregulated in head and neck squamous cell carcinoma[18] and associated metastasis and the Ki-67-dependent cell proliferation through activating TCF4/β-catenin signaling in lung cancer.[17] The role of NIFK in CRC remain to be further explored.

In the present study, the role of NIFK in CRC was investigated. NIFK expression, association with patient prognosis, correlation with infiltration of immune cells and its correlated genes involved in signaling pathways were analyzed using bioinformatics method. Our results would contribute to that NIFK was used as a potential predictor to prognosis of CRC patients.

2. Materials and Methods

2.1. Data acquisition

The data of samples involved in our study were acquired from the TCGA database (https://portal.gdc.cancer.gov) with 788 samples of rectal Cancer (MSK, Nature Medicine 2022), 594 samples of colorectal Adenocarcinoma (TCGA, PanCancer Atlas) and 275 samples of colorectal Adenocarcinoma as well as 41 normal samples(TCGA). 270 cases of colorectal Adenocarcinoma with survival data were available from TCGA. Two data packets of GSE106582 (194 samples) and GSE117606 (208 samples) were downloaded from GEO database (https://www.ncbi.nlm.nih.gov/gds).

2.2. Samples collected

102 CRC tissue samples (38 rectal cancer and 64 colon cancer) with clinical features including gender, age, tumor size, tumor-node-metastasis stage, degree of differentiation and regional lymph node metastasis and 30 para-carcinoma tissue samples (10 rectum and 20 colon) were collected in the Zhuzhou Hospital Affiliated to Xiangya School of Medicine, Central South University with informing consent according to our previous study.[19] This study was approved by the ethics committee of the he Affiliated Zhuzhou Hospital Xiangya Medical College CSU (No.: 2018K0327), and all patients signed an informed consent form.

2.3. Analysis of NIFK expression and its correlation with prognosis of patients in CRC

Pan-carcinoma analysis of NIFK expression was obtained from TIMER2.0 (http://timer.comp-genomics.org/). The expression of NIFK and poathological stage Plot in Colon adenocarcinoma (COAD), Rectal adenocarcinoma (READ) and normal control tissues from TCGA were analyzed using GEPIA (http://gepia.cancer-pku.cn/detail.php?gene=NIFK). The Survival plots were performed with overall survival, median, 50% of both cutoff-high and low along with 95% confidence interval from GEPIA.

2.4. Analysis of gene expression with immune infiltration

The correlation of gene expression with immune infiltration was analyzed using TIMER2.0 (http://timer.comp-genomics.org/). Gene expression with T cell CD8+, T cell CD4+, Tregs, B cell.

2.5. Differential gene and correlation analysis

Differential gene analysis was performed in 194 samples including 38 high and 38 low NIFK expression from data GSE106582 using GEO2R (R script, R 3.2.3). 77 samples of CRC were grouped as High and low NIFK expression based on median. Correlated genes of NIFK in CRC samples were analyzed using TIMER2.0 and cBioPortal (http://www.cbioportal.org/).

2.6. Kyoto encyclopedia of genes and genomes (KEGG) pathway gene set enrichment analysis

Gene set enrichment analysis (GSEA) was performed for KEGG pathway set. GSEA software (version 3.0) (http://software.broadinstitute.org/gsea/index.jsp) was used for high and low NIFK expression. The GMT Set to evaluate related pathways and molecular mechanisms. Based on the gene expression profile and phenotypic grouping, the minimum gene set was set as 5, the maximum gene set as 5000, 1000 times of resampling. *P* value of < .05 (as needed) and an FDR of <0.25 (as needed) were considered statistically significant according to Sangerbox(http://www.sangerbox.com/).

2.7. mRNA expression assayed by Real-time quantitative PCR (qPCR)

NIFK expression in 30 CRC (9 rectum and 21 colon) and 20 para-carcinoma tissue (6 rectum and 14 colon) samples was detected by qPCR. Total RNA were extracted using Trizol kit(Qiagen) for reverse transcription. After then, qRT-PCR was performed using a SYBR green PCR kit (Qiagen) on the iCycler iQ (Bio-RAD) according to our previous study.[19] The reaction program including pre-denaturation at 95°C for 10min as well as denaturation at 95°C for 10 seconds, annealing at 60°C for 30 seconds, extension at 72°C for 30 seconds involved in 45 cycles. β-actin was used to normalize target genes, the relative expression of which was obtained using 2 - Δ Δ CT method. The primers of β-actin were forward GGCACTCTTCCAGCCTTCC and reverse GAGCCGCCGATCCACAC. The primers of NIFK were forward CCTACCTAACCTACTTGACGAAACC and reverse CACTGATGGATATGATGGCTGC.

2.8. Immunohistochemistry (IHC)

Protein expression and localization of NIFK were analyzed in all the samples collected from our hospital by IHC staining based on our previous study.[19] A mouse-anti NIFK monoclonal primary antibody (ab244216, Abcam, UK) was used to identified NIFK protein and then marked by second antibody conjugated with horseradish peroxidase (HRP). The nucleus was counterstained using hematoxylin. The slices of samples were then observed under a microscope and 5 fields are randomly were selected for analysis NIFK expression. The expression of

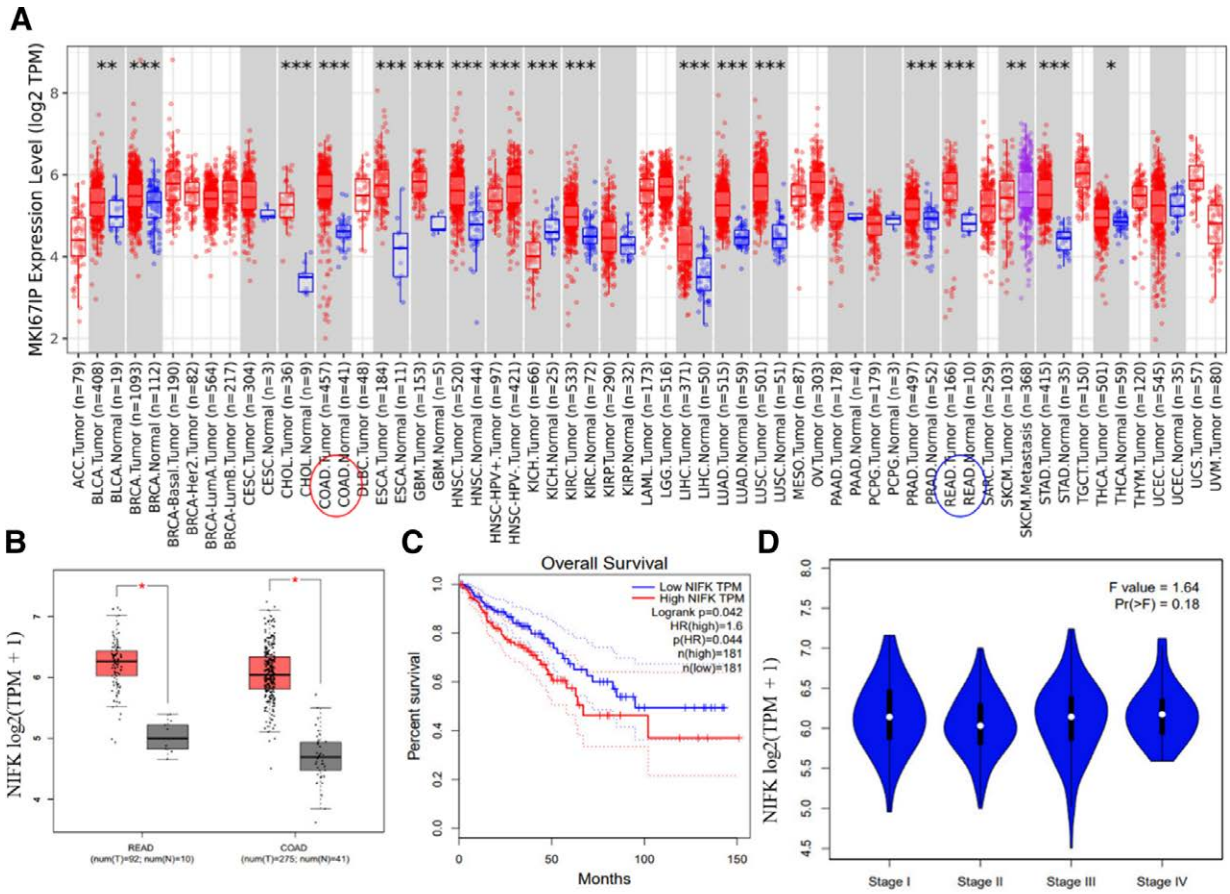

Figure 1. Expression of NIFK in tumor tissues and normal tissues along with survival of patients with colorectal cancer. (A) The expression of NIFK in tumor tissues and normal tissues based on analysis of samples using TIMER2.0. *P<.05, **P<.01, ***P<.001 compared with normal tissue. (B) NIFK expression tumor tissue of colorectal cancer and normal tissue analyzed using GEPIA. *P<.05 compared with normal tissue. (C) Survival analysis of CRC patients with high NIFK expression and low expression. (D) NIFK expression within the stage progress of CRC. CRC = colorectal cancer, NIFK = Nucleolar protein interacting with the FHA domain of MKI67.

NIFK was indicated by the average optical density analyzed using Image-Pro Plus 6.0 (USA).

2.9. Cells culture and cell experiment

Colon cancer cell line HCT 116 was cultured in McCoy 5A medium supplemented with 10% FBS and 1% penicillin and streptomycin at 37°C, 5%CO_2.

For cell proliferation assay, 2×10^3 cells per well were planted into a 96-well plate and cultured overnight, then the cells were transfected with NIFK down expression lentivirus (NIFK-KD) and negative control (NC) and cultured for another 24h, 48h and 72h. Next, 100 μL cck8 was added to each well and cultured at 37°C for 2h. The absorption value OD 450 of each well was determined by a microcoder.

For cell migration measurement, transwell was used according to the product introduction. The cells transfected with NIFK-KD or NC were seeded in the upper well in FBS free medium, the down well contained complete medium. The cells were allowed to migrate for 48h and then stained by Crystal violet as well as images of which were taken and analyzed.

2.10. Statistical analysis

SPSS 20.0 software (SPSS, Inc., Chicago, IL) was used for data analysis. The data are presented as mean ± SD from at least 3 independent tests. Differences among 3 and more groups were used One-way ANOVA. $P < 0.05$ is considered statistically significant.

3. Results

3.1. NIFK expression in cancer tissues and CRC tissues in different stages as well as its correlation with survival of patients with CRC and cell proliferation and migration.

As showed in Figure 1A, high expression of NIFK was found in bladder urothelial carcinoma(BLCA), breast invasive carcinoma (BRCA), cholangiocarcinoma(CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC)-HPV-, kidney renal clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD) and thyroid carcinoma (THCA). NIFK expression was significantly upregulated in tumor of COAD and READ compared with normal tissue was confirmed in data obtained from TCGA (Fig. 1B). Extended survival was found in CRC patients with low NIFK (n = 181) expression compared with those with high NIFK expression (P<.05) (n = 181) (Fig. 1C). NIFK expression (TPM) tended to be increased with the stage progress (Fig. 1D).

The mRNA expression of NIFK in tumor of CRC and para-carcinoma tissue samples was also assayed by qPCR and results of which was showed in Figure 2A. NIFK showed higher

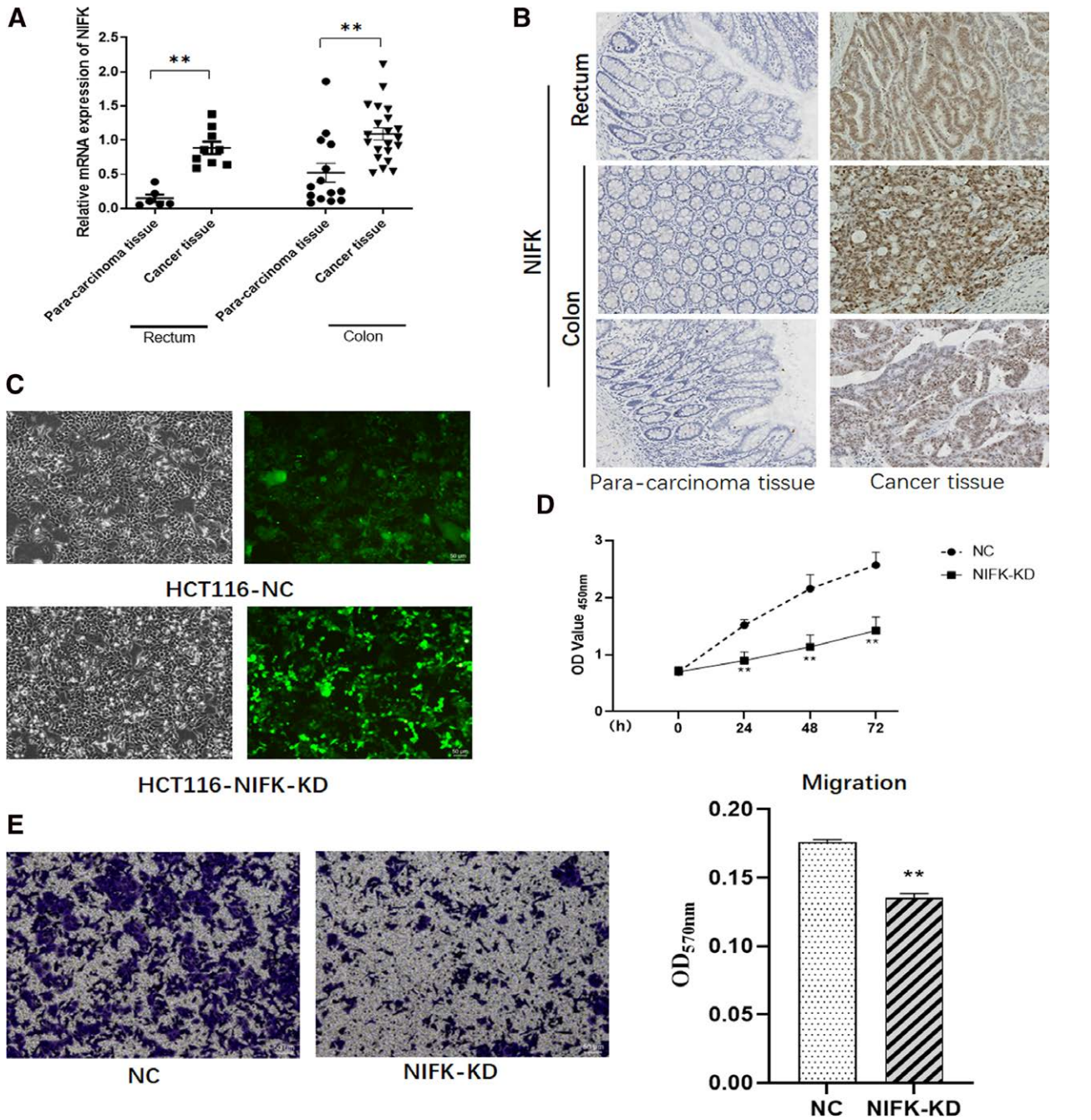

Figure 2. NIFK role in colorectal cancer. (A) mRNA in rectal cancer tissue (n = 9) and para-carcinoma tissue (n = 6) as well as colon cancer tissue (n = 21) and para-carcinoma tissue (n = 14). ***P*<.01 compared with para-carcinoma tissue. (B) Protein expression of NIFK in cancer tissue and para-carcinoma tissue assayed by immunohistochemistry. (C) Images of HCT 116 cells were transfected with NIFK down expression (NIFK-KD) and negatived control (NC) lentivirus. (D) Cell proliferation of HCT 116 cells transfected with NIFK-KD and NC was assayed using CCK8. (E) Migration of HCT 116 cells transfected with NIFK-KD and NC was detected using Transwell. ***P*<.01 compared with NC. n = 3. NIFK = Nucleolar protein interacting with the FHA domain of MKI67.

expression in tumor tissue of CRC samples(rectum and colon) than that in para-carcinoma tissue with significant differences (*P*<.05). It was similar to gene expression, protein expression of NIFK in CRC samples was significantly increased compared with that in para-carcinoma tissue (Fig. 2B, Table 1, Table 2). There was no significant correlation of NIFK expression with clinicopathology of rectal cancer which may be due to small sample size in the present study (Table 3). It was worth noting that high NIFK expression in colon cancer tissues was positively correlated with tumor size, differentiation, tumor-node-metastasis stage, N stage and M stage ($P < .05$) significantly, but had no significant correlation with gender, age, T stage of patients and carcinoembryonic antigen (Table 4). Moreover, there are no significant difference between NIFK expression and factors that may influence patient prognosis, such as age, gender, tumor size, tumor stage and carcinoembryonic antigen (Table 2). There were 11 cases of distant metastasis including 7 cases of liver metastasis involved 6 cases with positive NIFK expression, 1 case of liver and lung combined metastasis as well as 3 cases of abdominal implantation metastasis which were all expressed NIFK positively in colon cancer samples. Among rectal cancer samples, 2 cases of liver and lung combined metastasis which were all expressed NIFK positively. Knock down expression of NIFK suppressed cell proliferation and migration of colon cancer cells (Fig. 2C–E) which may partly validate that NIFK serves as an oncogene in colon cancer.

Table 1

Protein expression in Rectal cancer and paracancer tissue.

		NIFK			
	n	Low	High	χ^2	*P*
Rectal cancer	38	12	26	5.774	.016
Paracancer	10	8	2		

NIFK = Nucleolar protein interacting with the FHA domain of MKI67.

Table 2

Protein expression in Colon cancer and paracancer tissue.

		NIFK			
	n	Low	High	χ^2	*P*
Colon cancer	64	27	37	11.197	.001
Paracancer	20	17	3		

NIFK = Nucleolar protein interacting with the FHA domain of MKI67.

Table 3

Correlation of NIFK expression with clinicopathology of Rectal cancer.

		NIFK			
Clinicopathological index	n	Low	High	χ^2	*P*
Gender					
Male	25	9	16	0.198	.656
Female	13	3	10		
Age (yr old)					
≤60	15	6	9	0.297	.586
>60	23	6	17		
Tumor size (cm)					
<5	23	8	15	0.029	.866
≥5	15	4	11		
Differentiation					
High	11	3	8	0.662	.718
Medium	22	8	14		
Low	5	1	4		
TNM stage					
I	10	6	4	6.037	.110
II	6	1	5		
III	20	5	15		
IV	2	0	2		
T stage					
$T_1 + T_2$	14	6	8	0.609	.435
$T_3 + T_4$	24	6	18		
N stage					
N_0	16	7	9	1.895	.169
N_{1+2}	22	5	17		
M stage					
M0	36	12	24	1.569	.210
M1	2	0	2		
CEA (ng/mL)					
<5	27	10	17	0.561	.454
≥5	11	2	9		

CEA = carcinoembryonic antigen, NIFK = Nucleolar protein interacting with the FHA domain of MKI67, TNM = tumor-node-metastasis stage.

3.2. Correlation of NIFK with immune cell infiltration

To explore the role of NIFK in immunology, the correlation of NIFK with immune cell infiltration was analyzed in our study. As showed in Figure 3, The correlation of NIFK with T cell CD8+, T cell regulatory (Tregs), Neutrophil, macrophage in COAD was significant ($P<.05$). And The correlation of NIFK with Tregs in READ was significant ($P<.05$).

Table 4

Correlation of NIFK expression with clinicopathology of Colon cancer.

		NIFK			
Clinicopathological index	n	Low	High	χ^2	*P*
Gender					
Male	39	18	21	0.198	.656
Female	25	9	16		
Age (yr old)					
≤60	26	9	17	1.029	.310
>60	38	18	20		
Tumor size (cm)					
<5	25	16	9	8.003	.005
≥5	39	11	28		
Differentiation					
High	16	9	7	7.880	.019
Medium	32	16	16		
Low	16	2	14		
TNM stage					
I	9	4	5	9.428	.024
II	25	15	10		
III	19	7	12		
IV	11	1	10		
T stage					
$T_1 + T_2$	11	6	5	0.332	.564
$T_3 + T_4$	53	21	32		
N stage					
N_0	38	20	18	4.183	.041
N_{1+2}	26	7	19		
M stage					
M0	53	26	27	4.440	.035
M1	11	1	10		
CEA (ng/mL)					
<5	36	15	21	0.009	.924
≥5	28	12	16		

CEA = carcinoembryonic antigen, NIFK = Nucleolar protein interacting with the FHA domain of MKI67, TNM = tumor-node-metastasis stage.

3.3. Genes correlated with NIFK

The genes correlated with NIFK in COAD and READ were analyzed according to cBioPortal involved samples from TCGA. The expression of DARS correlates with NIFK(MKI67IP) in COAD (Fig. 4A) and READ (Fig. 4C) with value of the correlation coefficient more than 0.6. Similarly in COAD and READ, NKRF correlated with NIFK positively (Fig. 4B and D). It was noting that high expression of both DARS and NKRF was not good for CRC patient prognosis (Fig. 4E–G). Moreover, the expression of DARS and NKRF was increased in tumor tissue of COAD and READ compared with normal samples (Fig. 4F–H). In addition, the expression of DARS and NKRF was upregulated in tumor of BRCA, CHOL, HNSC-HPV-, LIHC, LUAD, LUSC and STAD significantly compared with normal tissues (Fig. 4I–J). DARS and NKRF were chosen for further analysis based on coefficient of association (rho), intersection of COAD and READ, correlation with survival of CRC patients and differential expression between tumor and normal tissues.

3.4. The role of DARS and NKRF in immune cell infiltration

The correlation of DARS and NKRF with immune cell infiltration was further investigated, and results were showed in Figure 5. DARS significantly correlated with CD8 + T cell, CD4 + T cell, macrophage in COAD while with B cell, CD8 + T cell, macrophage, Neutrophil in READ ($P<.05$). Besides to correlate with CD4 + T cell and macrophage in COAD, NKRF was correlated with CD8 + T cell and Neutrophil significantly.

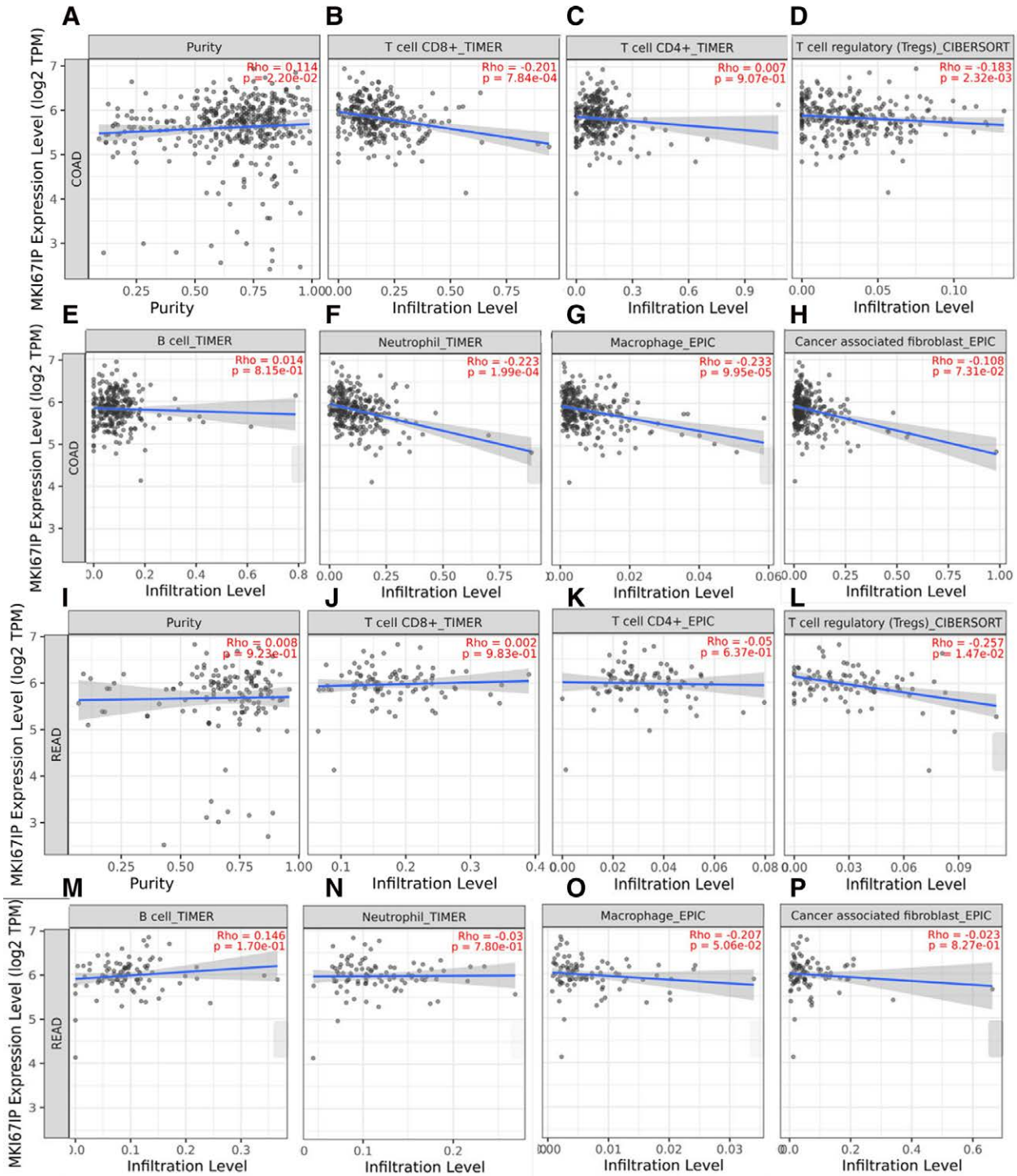

Figure 3. Correlation of NIFK(MKI67IP) with immune cell infiltration in colorectal cancer. (A) Immune cells purity in Colon adenocarcinoma (COAD). (B) Correlation of NIFK with CD8 + T cell in COAD. (C) Correlation of NIFK with CD4 + T cell in COAD. (D) Correlation of NIFK with Tregs in COAD. (E) Correlation of NIFK with B cell in COAD. (F) Correlation of NIFK with neutrophil in COAD. (G) Correlation of NIFK with macrophage cell in COAD. (H) Correlation of NIFK with Cancer associated fibroblast in COAD. (I) Immune cells purity in rectum adenocarcinoma (READ). (J) Correlation of NIFK with CD8 + T cell in READ. (K) Correlation of NIFK with CD4 + T cell in READ. (L) Correlation of NIFK with Tregs in READ. (M) Correlation of NIFK with B cell in READ. (N) Correlation of NIFK with neutrophil in READ. (O) Correlation of NIFK with macrophage cell in READ. (P) Correlation of NIFK with Cancer associated fibroblast in READ. NIFK = Nucleolar protein interacting with the FHA domain of MKI67.

3.5. Differential genes associated with NIFK in CRC and enrichment pathways

The differential genes between NIFK high expression and low expression samples of CRC involved in GE106582 (Fig. 6A and B) and enrichment pathways in KEGG were also analyzed. As showed in Figure 6C–D, NIFK involved in KEGG calcium signaling pathway and associated with many genes in the above-mentioned pathway. NIFK was also correlated with KEGG regulation of autophagy (Fig. 6E) and vasopressin regulated water reabsorption (Fig. 6G), as related genes were showed in Figure 6F and H. It was worth noting that NIFK associated with cell cycle, P53 and Wnt signaling pathways (Fig. 6I).

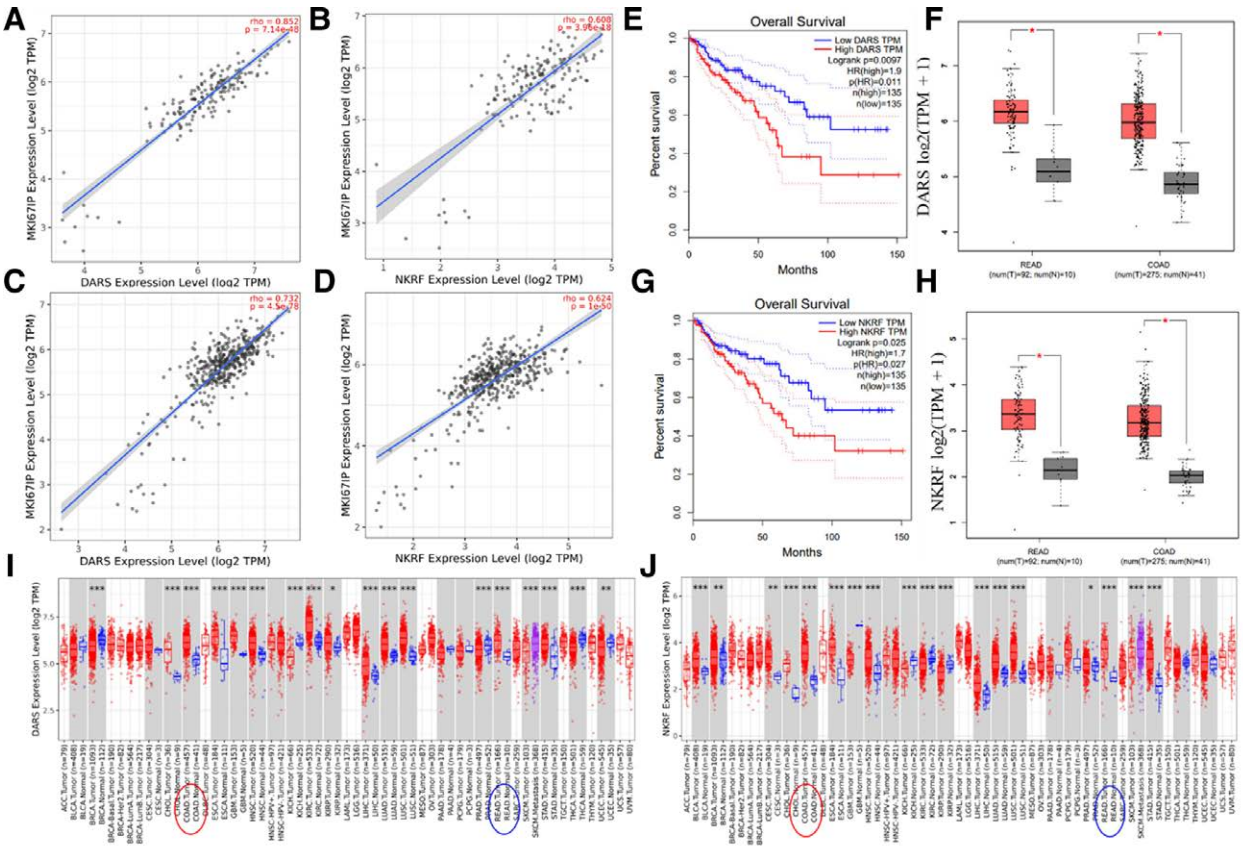

Figure 4. NIFK correlated DARS and NKRF expression and associated with survival in colorectal cancer. (A) Correlation of NIFK with DARS in Colon adenocarcinoma (COAD). (B) Correlation of NIFK with NKRF in Colon adenocarcinoma (COAD). (C) Correlation of NIFK with DARS in rectum adenocarcinoma (READ). (D) Correlation of NIFK with NKRF in rectum adenocarcinoma (READ). (E) Correlation of DARS expression with survival of patients with colorectal cancer. (F) DARS expression in colorectal cancer analyzed using GEPIA. *P<.05 compared with normal tissue. (G) Correlation of NKRF expression with survival of patients with colorectal cancer. (H) NKRF expression in colorectal cancer analyzed using GEPIA. *P<.05 compared with normal tissue. (I) The expression of DARS in tumor tissues and normal tissues based on analysis of samples using TIMER2.0. *P<.05, **P<.01, ***P<.001 compared with normal tissue. (J) The expression of NKRF in tumor tissues and normal tissues based on analysis of samples using TIMER2.0. *P<.05, **P<.01, ***P<.001 compared with normal tissue. NIFK = Nucleolar protein interacting with the FHA domain of MKI67.

Signaling pathways enrichment analysis was performed involved in NIFK with its correlated genes from cBioPortal, and results were showed in Figure 7A. NF-kappa B signaling, primary immunodeficiency, inflammatory bowel disease, mTOR signaling and CRC pathways enrichment involved in differential genes between NIFK high expression and low expression samples of GE106582 were analyzed in our study. Besides intestinal immune network for IgA production, Wnt, PIK3-Akt, NF-kappa B signaling pathways were involved in both NIFK with its correlated genes and differential genes between NIFK high expression and low expression CRC samples (Fig. 7B).

4. Discussion

Poor prognosis of patients remains a challenge to therapy in CRC currently. As showed in many studies, tumor initiation, progress, metastasis correlated with immune-related tumor microenvironment[20,21] and it was increasingly recognized that tumor development, progression and response to therapy were correlated with immune landscape.[5] Prognostic biomarkers, especially immune-related markers play an important role in improving survival of CRC patients.

As one of RNA binding proteins, NIFK plays a role in tumor cell proliferation and metastasis, which may be associated with immune-related NETs in cancer.[17,18] In the present study the role of NIFK in CRC were explored through bioinformatics analysis of data obtained from TCGA, GEO and samples collected in our institution. First of all, NIFK expression in CRC tumor tissues and normal tissue was investigated. Our results indicated the expression of NIFK in tumor tissues was significantly increased compared with normal samples, which was positively correlated with tumor progress. The results of cell experiment in vitro confirmed that down-regulated NIFK expression inhibited cell proliferation and migration of CRC cells which may suggest that NIFK serves as an oncogene in CRC. And NIFK expression results were consistent in all the data (sample) from database and our institution. Then survival of CRC patients in NIFK high and low expression groups were analyzed, results of which showed that CRC patients with high NIFK expression have poor survival compared with those with low NIFK expression. The data suggested that NIFK may be associated with poor prognosis of CRC patients.

Tumor immune cell infiltration including T cell, macrophage and neutrophils also contributed in tumor occurrence and development. Tumor-infiltrating lymphocytes (TILs), tumor-associated macrophages and tumor-associated neutrophils are involved in CRC.[9] Correlation of NIFK with immune cell infiltration was then explored in our study. It was observed that NIFK negatively correlated with T cell CD8+, Tregs, Neutrophil and macrophage significantly. The role of T cell CD8 + was complicated in cancer. CD8 + T cell killing may be helpful to suppress tumorigenesis in CRC.[22] In another study, blockade CD8 + T-cell infiltration into tumors may related with delayed tumor progression in CRC.[23] As

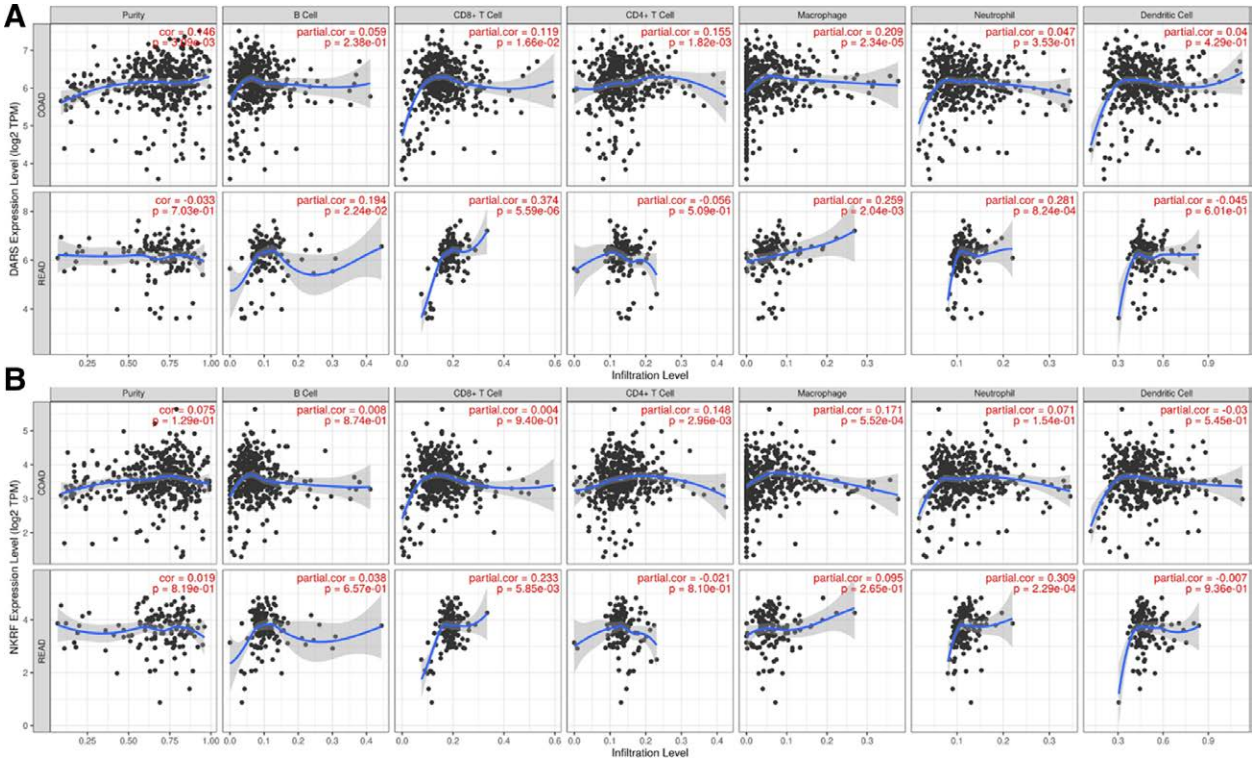

Figure 5. Correlation of DARS and NKRF with immune cell infiltration. (A) Correlation of DARS with CD8 + T cell, CD4 + T cell, Tregs, B cell, neutrophil, macrophage and Cancer associated fibroblast in Colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ). (B) Correlation of NKRF with CD8 + T cell, CD4 + T cell, Tregs, B cell, neutrophil, macrophage and Cancer associated fibroblast in Colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ).

one kind of immune cells associated with tumor microenvironment, Tregs contributed to prognoses of patients with cancer.[24] Neutrophil-albumin ratio may be a biomarker for prognosis of patients with CRC.[25] It was similar that neutrophil-to-lymphocyte ratio also play a role in metastatic CRC.[26] Moreover, NETs involved in Neutrophil play an important role in tumor development and metastasis.[27] Macrophages including M1/M 2 related with tumor microenvironment correlated with clinical outcomes of with patients.[28] T cell CD8 + and M1 macrophages might serve a potential biomarker to prognosis of patients with CRC.[28] It was suggested in our study that immune cell infiltration-related NIFK might be a potential biomarker to CRC patient prognosis.

To further investigate the role of NIFK in CRC, correlated genes of NIFK were involved in our study. As differential expression, correlation with NIFK, association with survival of CRC patients were considered, DARS and NKRF were filtrated for further investigation. DARS associated with hypomyelination with brainstem and spinal cord involvement and leg spasticity and was identified in CRC.[29] DARS was identified as a therapeutic target and novel prognostic marker for gastric cancers.[30] As a NF-κB Repressing Factor, NKRF mediates transcriptional repression of certain NK-kappa-B responsive genes.[31] NKRF combined with NF-κB pathway to confer chemoresistance of lung cancer cell.[32] Moreover, NKRF was suppressed in gastric cancer to promote tumor progression.[33] Our results showed that DARS correlated with CD8 + T cell, CD4 + T cell, macrophage and Neutrophil in CRC. There was significant correlation of NKRF with CD8 + T cell, CD4 + T cell and macrophage in CRC. It was suggested that NIFK and its correlated genes as DARS and NKRF might contribute biomarkers for prognosis of CRC patients.

Signaling pathways analysis of NIFK along with its correlated genes as DARS and NKRF was performed in the present study. Wnt, PI3K-Akt, NF-κB signaling and Intestinal immune network for lgA production were involved from analysis of either NIFK and its correlated genes or high NIFK expression and low NIFK expression samples. Wnt family members associated with cell survival, angiogenesis and abnormal vascular development, and activation of wnt pathway could drive CRC initiation and development.[34] Tumor associated macrophages closely related to tumor initiation, development and metastasis, which could release many kinds of cytokines such as IL-1β, CXCL-8, and CXCL-12 to activate Wnt target genes including c-Myc and c-Jun.[35] In addition, CXCL-8 and CXCL-12 correlated with the invasion of CRC cells and could stimulate the angiogenesis via activation Wnt/β-catenin signaling pathway and through regulating Wnt signaling pathway could inhibit CRC angiogenesis.[36] NF-κB signaling pathway correlated with cell proliferation, apoptosis, angiogenesis and metastasis in CRC.[36] PI3K/AKT activation in CRC could lead to increasing cell survival, hyperplasia, and cell proliferation.[37] Target to PI3K/AKT signaling pathway could contribute to improve therapeutic effect in CRC.[18,38] And most importantly, Wnt, PI3K-Akt and NF-κB signaling pathways might associate with tumor immune microenvironment in CRC. Therefore, NIFK with its correlated genes such as DARS and NKRF were suggested as inanition, progress, metastasis and tumor immune microenvironment in CRC.

4.1. Conclusion

In conclusion, NIFK might be a biomarker associated with poor prognosis of CRC patients, and it would be a potential target for CRC therapy. In particular, it was needed to perform experiment to validate the role of NIFK related with immune in CRC and in more samples of both colon cancer and rectal cancer in our further study.

Author contributions

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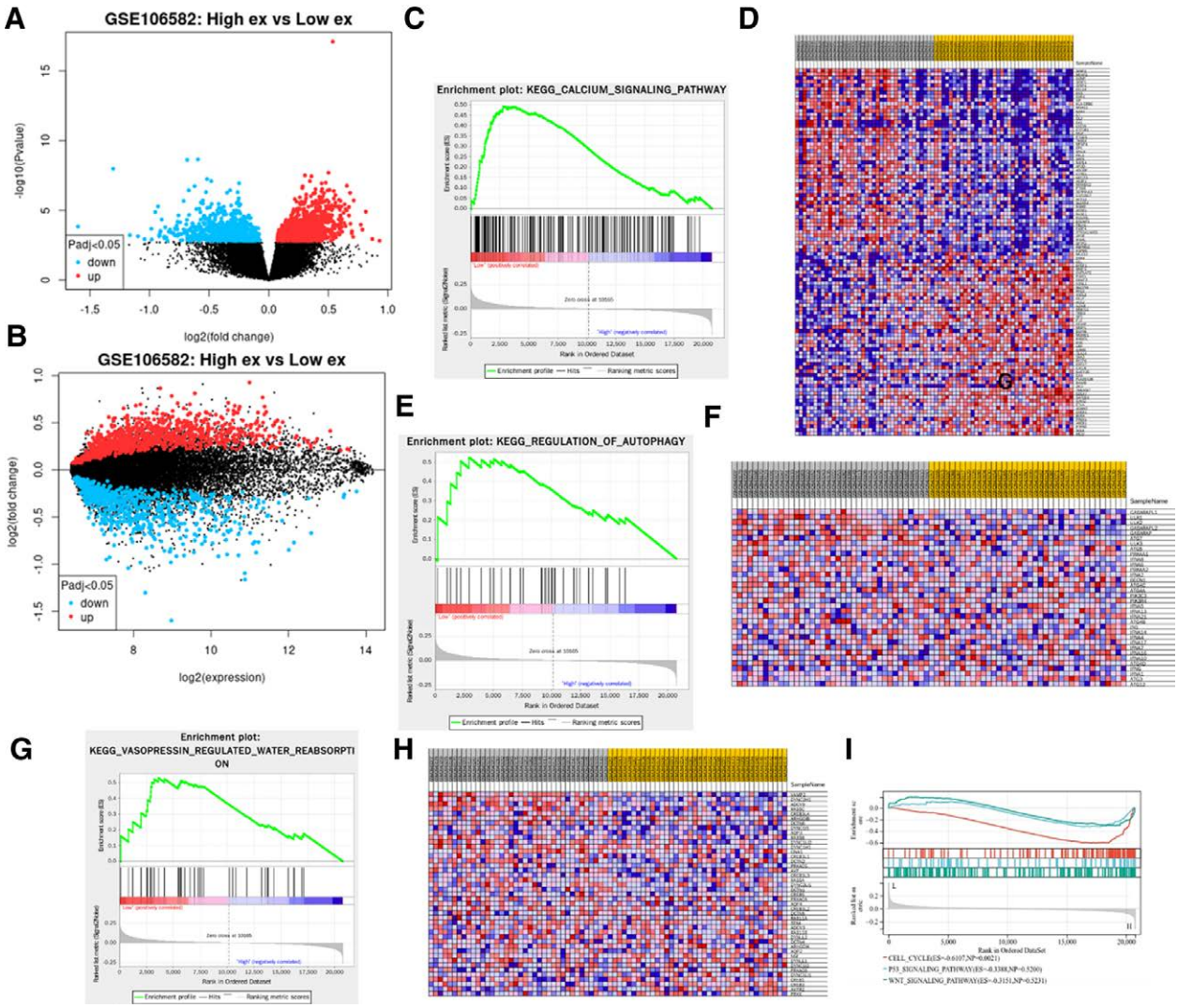

Figure 6. Differential genes associated with NIFK in colorectal cancer and KEGG enrichment pathways. (A and B) The differential genes between NIFK high expression and low expression samples of CRC involved in GE106582. (C) NIFK involved in KEGG calcium signaling pathway. (D) Genes associated with KEGG calcium signaling pathway. (E) NIFK involved in KEGG regulation of autophagy pathway. (F) Genes associated with KEGG regulation of autophagy pathway. (G) NIFK involved in KEGG vasopressin regulated water reabsorption pathway. (H) Genes associated with KEGG vasopressin regulated water reabsorption pathway. (I) NIFK associated with cell cycle, P53 and Wnt signaling pathways. CRC = colorectal cancer, NIFK = Nucleolar protein interacting with the FHA domain of MKI67.

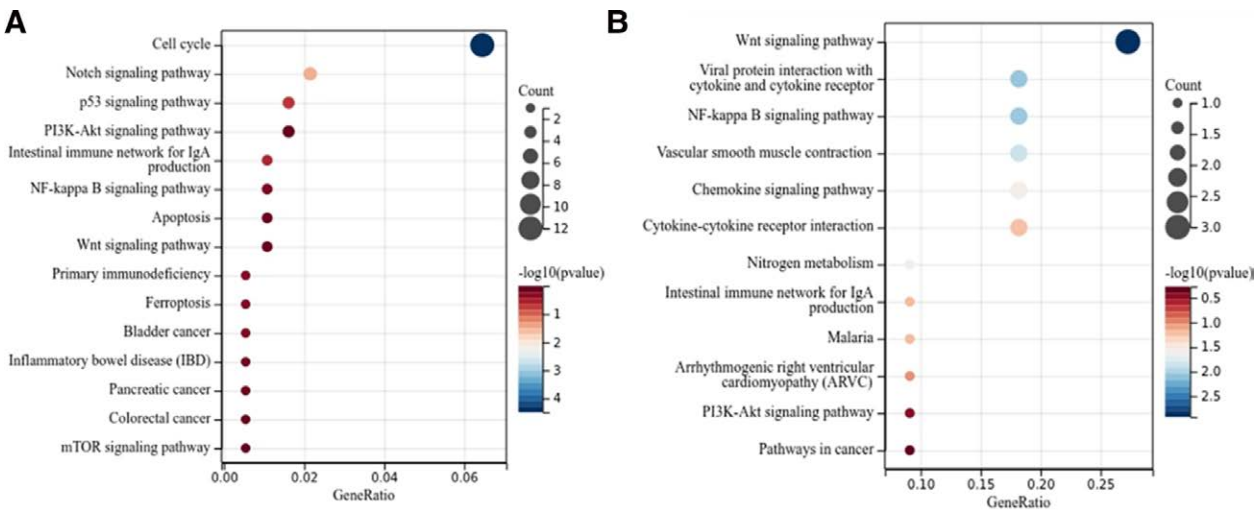

Figure 7. Signaling pathways enrichment involved in NIFK and its correlated genes. (A) Signaling pathways enrichment involved in NIFK with its correlated genes from cBioPortal. (B) Signaling pathways enrichment involved in differential genes between NIFK high expression and low expression samples of GE106582. NIFK = Nucleolar protein interacting with the FHA domain of MKI67.

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