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Original Article

Comprehensive analysis to long non-coding RNA-mediated high expression of GNG5 correlates with better prognosis and tumor immune infiltration of colon carcinoma

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Abstract: Background: Colorectal cancer is the third most common cancer and the fourth leading cause of cancer deaths. Prognosis is poor. The majority of patients are diagnosed with locally advanced or metastatic disease. Increasing evidence suggests G protein subunit gamma 5 (GNG5) play key roles in several types of human cancer. The key gating mechanisms in colorectal cancer remains unkown. Methods: In this study, pan-cancer analyses have been performed for GNG5's expression. Prognosis using The Cancer Genome Atlas and The Genotype-Tissue Expression data found that GNG5 are activated oncogenes in colorectal cancer. Noncoding RNAs play increasingly appreciated gene-regulatory roles and long noncoding RNAs contributing to GNG5 overexpression. They were identified by a combination in silico computational analyses. We identified candidate regulators controlling colon carcinoma survival analysis and correlation analysis. Results: The SNHG4/DRAIC-let-7c-5p axis was identified as the most progressive upstream lncRNA-related pathway of GNG5 in colorectal cancer. The GNG5 level was significantly negatively correlated with tumor immune cell infiltration, immune cell biomarkers, and immune checkpoint expression. Conclusions: Our findings elucidated that lncRNAs-mediated downregulation of GNG5 correlated with better prognosis and tumor immune infiltration in colorectal cancer.

Keywords: Colorectal cancer, prognosis, GNG5, lncRNA

Intruoduction

Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of cancer-related deaths due to distant metastases [1]. The 5-year overall survival for CRC patients without metastasis is 80-90%. The survival rate for patients with metastatic colorectal cancer is approximately 13% [2].

Postoperative adjuvant chemotherapy is the main adjuvant therapy for stage II-III patients. For locally advanced rectal cancer, the use of standard mFOLFOX6 regimen (oxaliplatin, calcium folinate, and fluorouracil) during preoperative neoadjuvant radiotherapy can improve the local response rate and PCR rate. It can improve the likelihood of radical resection and anal preservation. It does not improve the long-term prognosis. Intraperitoneal hyperthermic perfusion (HIPEC) had a certain effect on peritoneal metastasis of colorectal cancer. HIPEC could improve the survival of peritoneal metastasis of colorectal cancer [3]. It is not effective for patients with more peritoneal metastases and greater burden of metastatic tumors. For metastatic colorectal cancer, the advantages and disadvantages of first-line three-drug chemotherapy have been clarified, and high toxicity and subsequent drug selection difficulties are the bottleneck restricting three-drug therapy. There are currently biomarkers resistant to EGFR therapy such as HER2/MET amplification and ALK/ROS/NTRKs/RET fusion [4, 5]. NGS analysis reveals the role of HER2/PI3K/PTEN/RAS mutations and microsatellite instability (MSI) detected by PCR for treatment maintenance. To explore effective second-line target-

ing and immunotherapy schemes, exploring new effective targets and markers is a way to find effective treatment. Mechanisms for CRC metastasis and the therapeutic strategies are of paramount importance. It remains an urgent need to develop effective therapeutic targeting or seek promising prognostic biomarkers in CRC. The class G protein family is a group of proteins that plays a key role in cell division, diferentiation, and metastasis during embryonic development through direct the action of relevant G protein-coupled receptors [6, 7]. Several studies have found that G-protein family members have a strong influence on cancer progression. GNG7 is a member of the G Protein family proteins. It promotes renal clear cell carcinoma and esophageal cancer tumor progression through epigenetic silencing gene [8, 9]. The studies demonstrate that GNG4 function shows a high degree of correlation with liver cancer and colorectal cancer and is valuable for disease diagnosis and prognosis [10, 11]. In gastric cancer cells, GNG11 has shown to promote adhesion, migration, invasion, and survival of cancer cells [12]. The G-protein family contributes to tumor progression and can be used as biomarkers of disease diagnosis and treatment for tumors. The evidence has accumulated indicating that G protein family including GNG5 are widely expressed and play a role in tumor growth, invasion, angiogenesis and metastasis. In the literature, the examples of studies focusing on describing the role of GNG5 are limited in tumors.

GNG5 have a significant role in cellular biology, localizing on human chromosome 1p22. They have a significant role in cellular biology, localizing on human chromosome 1p22. They drive disease initiation and the progression of many diseases [13]. It plays an important role in regulating insulin secretion. It is used as a marker of melanosis coli related with cell apoptosis [14]. GNG5 encodes a member of G protein. It plays a key role in the mechanisms regulating G protein-coupled receptor (GPCR) signalling, involving cellular internalization and trafficking [15]. Previous studies have shown G-protein could induce diverse cellular responses including apoptosis in various cancers, such as murine colon adenocarcinoma and human melanoma cells [16, 17]. GNG5 has previously been shown to play an important role in several cancers, such as endometrial cancer and invasive ductal carcinoma of the breast, by regulating the secretion of e-cadherin through pathways including the Wnt signaling pathway [18].

It is widely documented that overexpression of GNG5 is associated with poor prognosis in various human cancer types, such as glimo and endometrial cancer. Immune signaling and immune synapse formation of GNG5 have been reported to affect various related human diseases. Functional analysis of GNG5 revealed that GNG5 were highly down regulated transforming growth factor beta (TGF-β) pathway. GNG5 are expressed on the immune cells' surface and can regulate immune signalling and effector functions [19]. The immunological role of GNG5 are involved in activation, migration, proliferation, and cytokine secretion of immune cells [20]. A report showed that GNG5 participate in malignant progression of gliomas. Tumor microenvironment play a critical role in glioma progression. These are reported to be related to the immune response process [21]. Regarding to the expression, prognosis, and mechanism of GNG5 in CRC, we have not witnessed comprehensive investigations. The connection between GNG5 and tumor immune infiltration in CRC continues to remain undetermined. The purpose of our study was to investigate the role of GNG5 in the prognosis and immunoinfiltration of colorectal cancer.

Materials and methods

TCGA data process and analysis

The mRNA expression data of 33 cancer types (ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LAML, LGG, LIHC, LUAD, LUSC, MESO, OV, PAAD, PCPG, PRAD, READ, SARC, SKCM, STAD, TGCT, THCA, THYM, UCEC, UCS, and UVM) were downloaded from TCGA database (https://genome-cancer.ucsc.edu/). After these data were normalized and differential expression analysis of GNG5 was performed using R package limma and ggplot2 [22]. *P* value <0.05 was considered as significant.

Candidate miRNA prediction

For target gene prediction miRNAs of GNG5 were predicted by several target gene prediction programs, consisting of starBase, PITA, NA22miRmap, microT, miRanda, PicTar, TargetS, canmetascape, and cytoscape. The pre-

dicted miRNAs that typically appear in more than two procedures were included in the analysis. These predicted miRNAs are considered as candidate miRNAs for GNG5.

StarBase database analysis

StarBase is an open-source database for investigating non-coding RNA interactions from CLIP-seq. We explored miRNA-related studies in the starBase (http://starbase.sysu.edu.cn/) [23]. Expression correlation analysis were performed using starBase for miRNA-GNG5, lncRNA-let-7c-5p or lncRNA-GNG5 in CRC. The expression level of let-7c-5p in CRC and normal controls was performed analyzed by starBase. The starBase database was used to predict the lncRNAs whose binding can be altered let-7c-5p.

GEPIA database analysis

Gene Expression Profiling Interactive Analysis GEPIA (http://gepia.cancer-pku.cn/) is a web-based tool for cancer and normal gene-expression profiling and containing high-throughput RNA sequencing data (TCGA and GTEx databases) [24]. GEPIA was used for determining GNG5 and lncRNA expression in various human cancer types. *P* value <0.05 is considered significant. Overall survival studies of common cancer types for GNG5 were analyzed by the gene expression profiling interactive analysis GEPIA. The prognostic values of candidate lncRNAs were assessed though GEPIA in CRC. Log rank *p* value <0.05 was considered as significant. The expression correlation of GNG5 with immune checkpoints in CRC was evaluated using GEPIA database. |R| >0.1 and *P* value <0.05 were set as selection criteria for identifying as significant.

Kaplan-Meier analysis

Xiantao Academic includes the TCGA database. It utilizes sequencing data and clinical information for online analysis. The Kaplan-Meier analysis of miRNAs in related gene sets were analyzed using the Xiantao platform (https://www.xiantao.love/). The survival analysis of let-7C-5P in CRC was performed using Xiantao platform. Log rank *P* value <0.05 was considered significant.

TIMER database analysis

Immune cell infiltration of TCGA primary tumors were downloaded from the TIMER web server (https://cistrome.shinyapps.io/timer/) [25]. The infiltration estimation results generated by the TIMER algorithm consist of 6 specific immune cell subsets, including B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells. We extracted infiltration estimates to assess the relationship between GNG5 expression levels and different immune cell subsets. The correlation between GNG5 and immune checkpoint expression in colorectal cancer was analyzed using TIMER. *P* value <0.05 was considered as significant.

Statistical analysis

The obtained data were analyzed using R software (version 4.2.1). The R package included ggplot2 [3.3.6], stats [4.2.1], and car. According to the characteristics of the data format, appropriate statistical methods were selected for statistics (stats package and car package). A survival package was used to test the proportional risk hypothesis. Fitted survival regression and the ggplot2 package was used for data visualization. The results are presented as the median and 95% confidence interval (CI). We used Kaplan-Meier curve by R software. Chi square and Logrank test were used for statistical analyses. The statistical significance was established at P<0.05. P<0.05 was considered as statistically difference.

Results

Pan-cancer analysis of GNG5 expression

To explore the role of GNG5 in CRC, we analyzed its expression in several human cancers. Compared with normal samples, we found that GNG5 was upregulated in 9 cancer types, including BRCA, COAD, CHOL, ESCA, GBM, READ, KIRP, PRAD, and LIHC, and was significantly downregulated in 3 cancer types, involving KICH, KIRC, and LUAD (**Figure 1**). We validated the expression of GNG5 in these cancer types using GEPIA database. To investigate the possible role of GNG5 in CRC, based on the Human Protein Atlas (HPA) database, we found that GNG5 was overexpressed in CRC tissues. Thdy were downexpressed in normal colon tissue by immunohistochemistry (IHC) staining obtained from the HPA (**Figure 2A** and **2B**).

The prognostic values of GNG5 in various cancer types

The overall survival analysis for GNG5 in BRCA (A), CHOL (B), LIHC (C), KIRC (D), KIRP (E), COAD

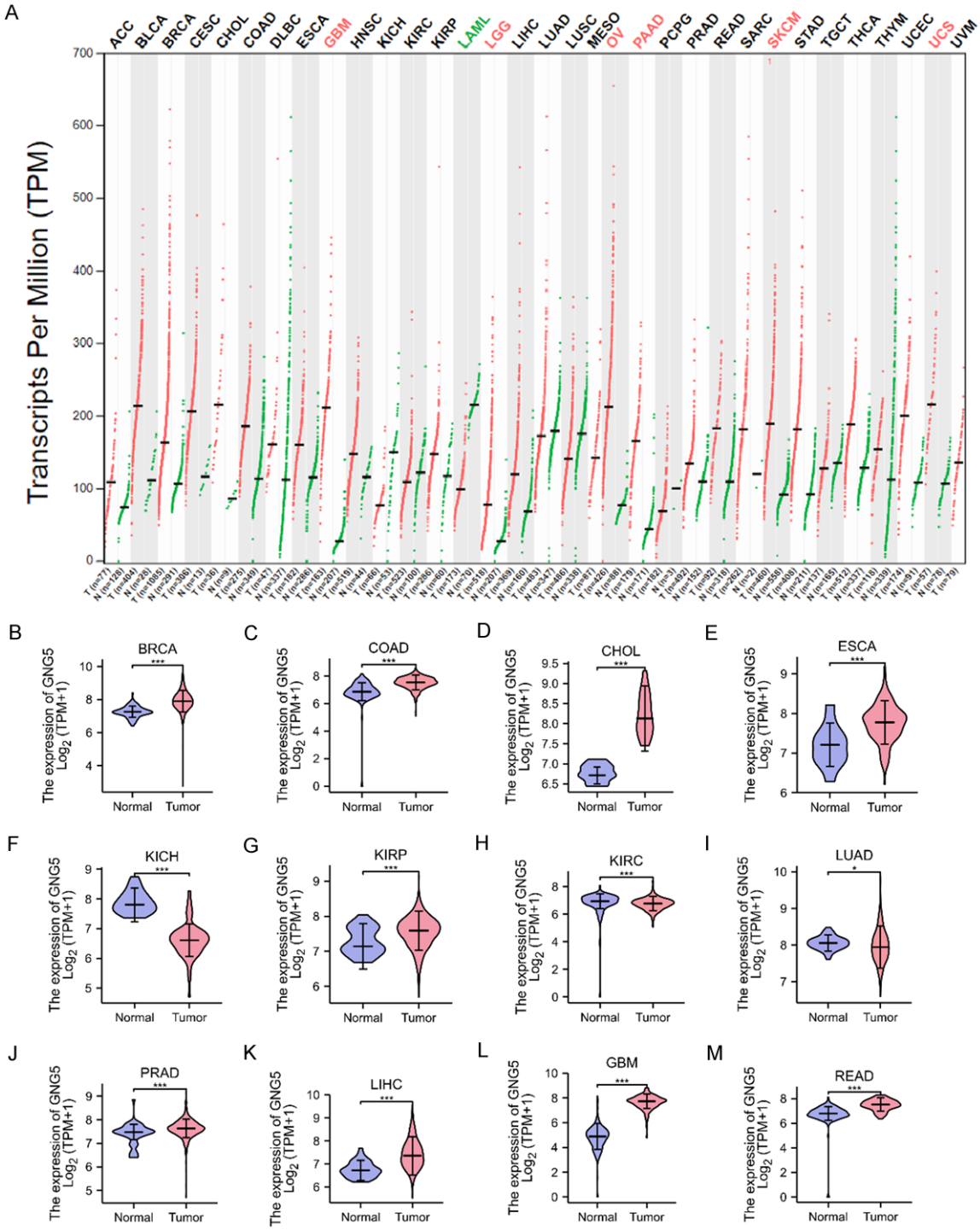

Figure 1. Expression analysis for GNG5 in multiple cancers (A). The expression of GNG5 in Pan-cancer analysis based on TCGA cancer and normal data (B-M). GNG5 expression in TCGA, BRCA (B), COAD (C), CHOL (D), ESCA (E), KICH (F), KIRP (G), KIRC (H), LUAD (I), PRAD (J), LIHC (K), GBM (L), and READ (M) tissues compared with corresponding TCGA and GTEx normal tissues. $^{*}p$ value <0.05; $^{**}p$ value <0.01; $^{***}p$ value <0.001.

(F), LUAD (G), GBM (H), KICH (I), PRAD (J), ESCA (K), and READ (L) was conducted. The results showed high expression of GNG5 in COAD indicated a better prognosis, but LIHC patients with higher expression of GNG5 had an unfavorable prognosis (**Figure 3**). The difference

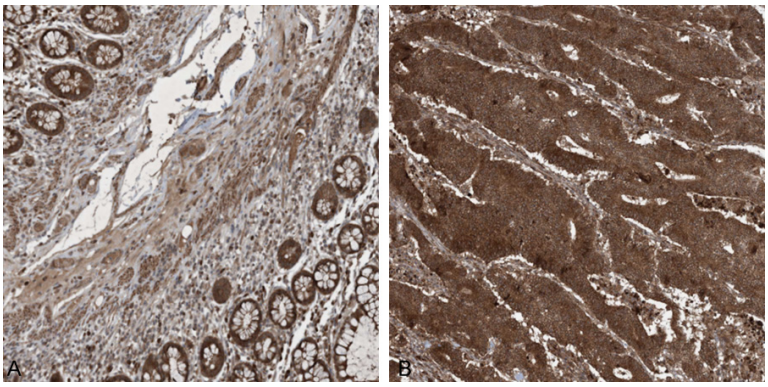

Figure 2. (A) Normal tissue and (B) tumor tissue by immunohistochemistry (IHC) staining 200×.

was not significant of GNG5 for predicting prognosis of patients in cancer types. Analysis of disease-free survival (RFS) of GNG5 determined by the GEPIA database in various human cancers showed no significant difference in RFS in colorectal cancer (**Figure 4**). GNG5 is utilized for the development of improved prognostic biomarkers in CRC patients.

Analysis of predicted upstream miRNAs of GNG5

It is widely acknowledged that ncRNAs are responsible for genes regulating expression. To examine how the modulation of some ncRNA would impact GNG5, we explored the predicted upstream miRNAs that could bind to GNG5 and found 20 miRNAs clusters. To improve visualization of the image, we modularized miRNA-GNG5 into regulatory network using cytoscape software (**Figure 5A**). As shown in **Figure 5B**, let-7C-5p expression was down-regulated in colon cancer, and high let-7C-5p expression was negatively correlated with patient prognosis (**Figure 5C**). Together, the mechanisms of miRNA-based gene regulation of the expression of target genes, negative correlation was found between miRNA and GNG5. We performed a correlation analysis based on the expression status. As listed in **Table 1**, GNG5 was significantly negatively correlated with let-7c-5p in CRC. There were no statistical expression relationships between GNG5 and the 19 predicted miRNAs. The expression and prognostic value of let-7c-5p in CRC were determined. An analysis of predicted miRNAs' targets showed that these genes were primarily involved in protein binding, cell adhesion, and cancer metastasis.

Prediction and analysis of upstream lncRNAs of let-7c-5p

StarBase database was used to predict the upstream lncRNA of let-7C-5P. A total of 17 possible lncRNAs were identified by lncRNA-let-7C-5p regulatory correlation analysis. We determined the expression levels of these lncRNAs in CRC using GEPIA. As shown in **Figure 6A**, **6B**, among all the 17 lncRNAs, only SNHG4 and DRAIC were significantly upregulated in CRC compared with normal controls. We assessed the prognostic value of the four lncRNAs in CRC. As suggested in **Figure 6C**, **6D**, higher DRAIC expression was associated with higher overall survival (OS) of CRC patients at 5 years (P = 0.02). Overexpressed SNHG4 indicated high OS of patients with CRC (P = 0.08). According to the Competing Endogenous RNA (ceRNA) hypothesis, two lncRNAs are correlated with LET-7C-5P or GNG5 in CRC. This was detected by starBase database, listed in **Table 2**. Survival analysis showed a correlation between DRAIC/SNHG4 expression and overall survival (OS) in CRC, DRAIC can be the most successful upstream lncRNAs of let-7c-5p/GNG5 axis in CRC.

GNG5 was positively correlated with immune cell infiltration in CRC

GNG5 has an immunoglobulin domain and the immune system plays a crucial role in the progression of colorectal cancer. As shown in **Figure 7A**, we observed significant differences immune cell infiltration immune cell infiltration level under various copy numbers of GNG5 in CRC. Analysis of GNG5 could yield clues to studying correlation of the function and mechanism. The correlation level of immune cell infiltration with the GNG5 expression was evaluated. As presented in **Figure 7B**, **7C**, The expression of GNG5 in CRC was positively correlated with B cells, CD8+ T cells, neutrophils, and dendritic cells, especially with B cells and CD8+ T cells.

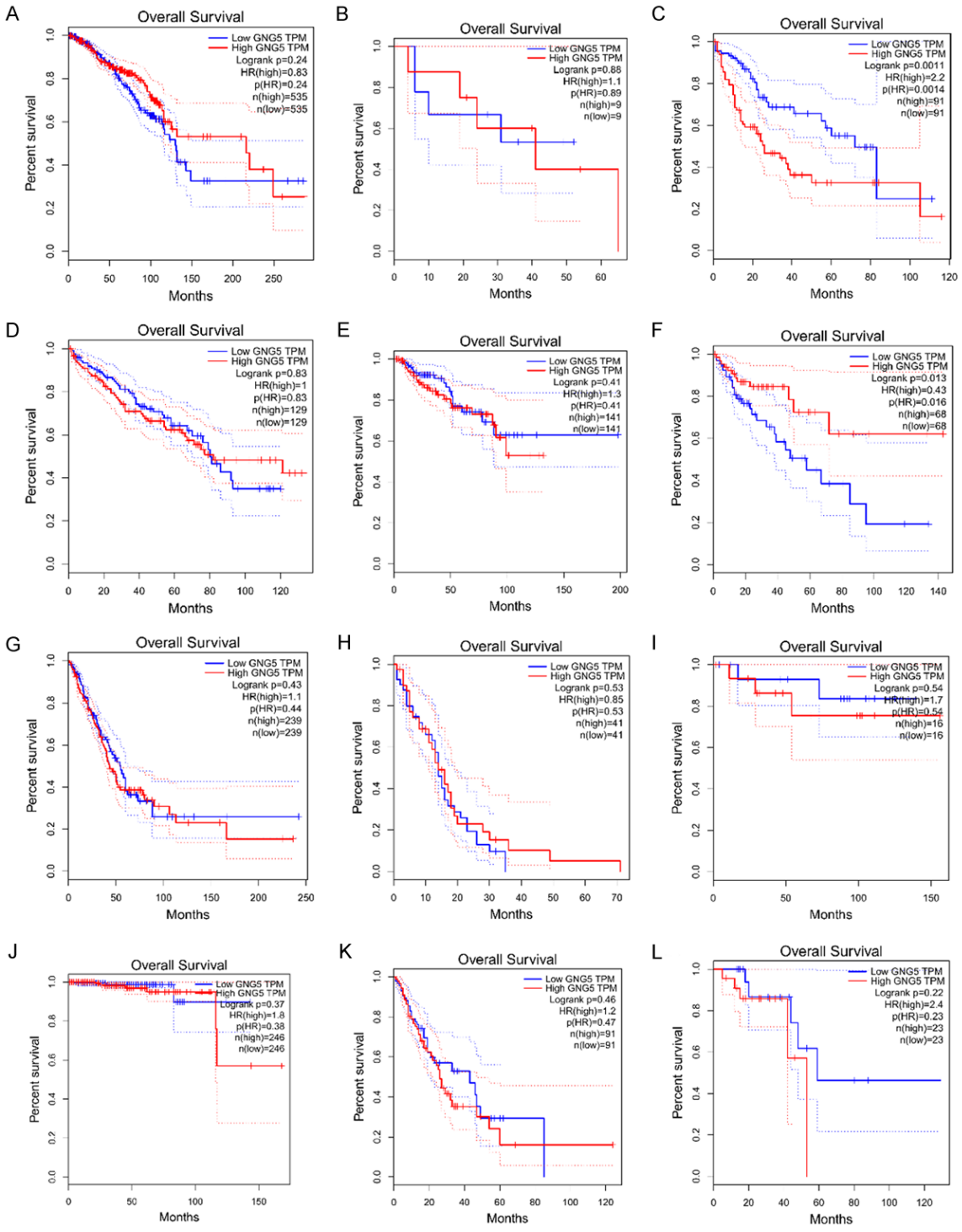

Figure 3. The overall survival (OS) analysis for GNG5 in various human cancers determined by GEPIA database. (A-L) The OS plot of GNG5 in BRCA (A), CHOL (B), LIHC (C), KIRC (D), KIRP (E), COAD (F), LUAD (G), GBM (H), KICH (I), PRAD (J), ESCA (K), and READ (L).

Correlation between GNG5 and expression of immune cell markers in CRC

We explored the roles of GNG5 in tumor immune. We analyzed the expression of GNG5 and correlations between the expression of any biomarkers of immune cells in CRC using GEPIA database. As listed in **Table 2**, GNG5 was negatively correlated with CD4+ T cell biomarker (CD4), M2 macrophage biomarker

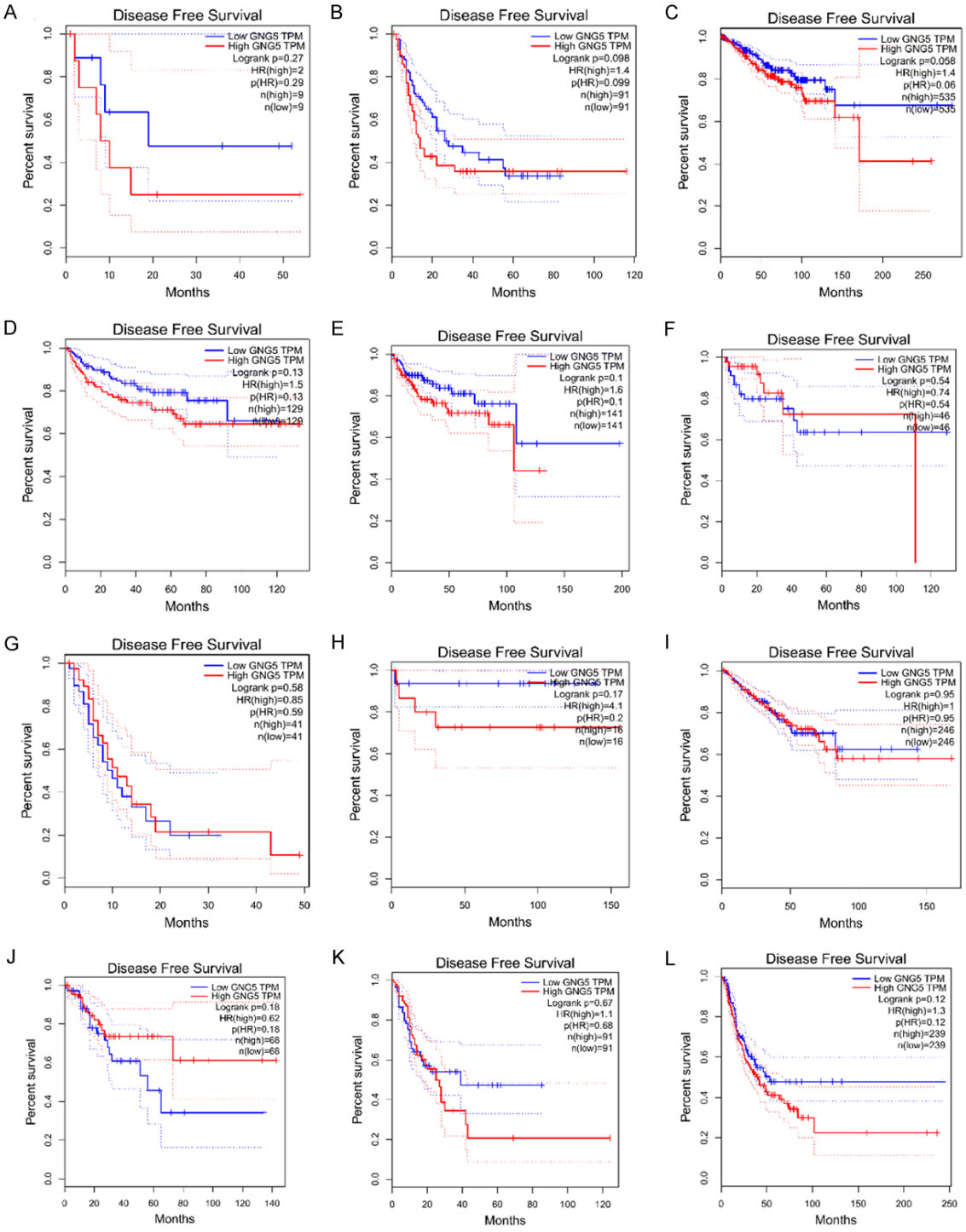

Figure 4. The disease-free survival (RFS) analysis for GNG5 in various human cancers determined by GEPIA database. (A-L) The RFS plot of GNG5 in CHOL (A), LIHC (B), BRCA (C), KIRC (D), KIRP (E), READ (F), GBM (G), KICH (H), PRAD (I), COAD (J), ESCA (K), and LUAD (L).

(CD163, VSIG4, MS4A4A), neutrophil biomarker (ITGAM), dendritic cell biomarker (NRP1, ITGAX) in CRC. These results suggest that GNG5 are involved in the down-regulation of immune cells.

Relationship between GNG5 and immune checkpoint in CRC

PD1/PD-L1 and CTLA-4 are important immune checkpoints that play a pivotal role in tumor

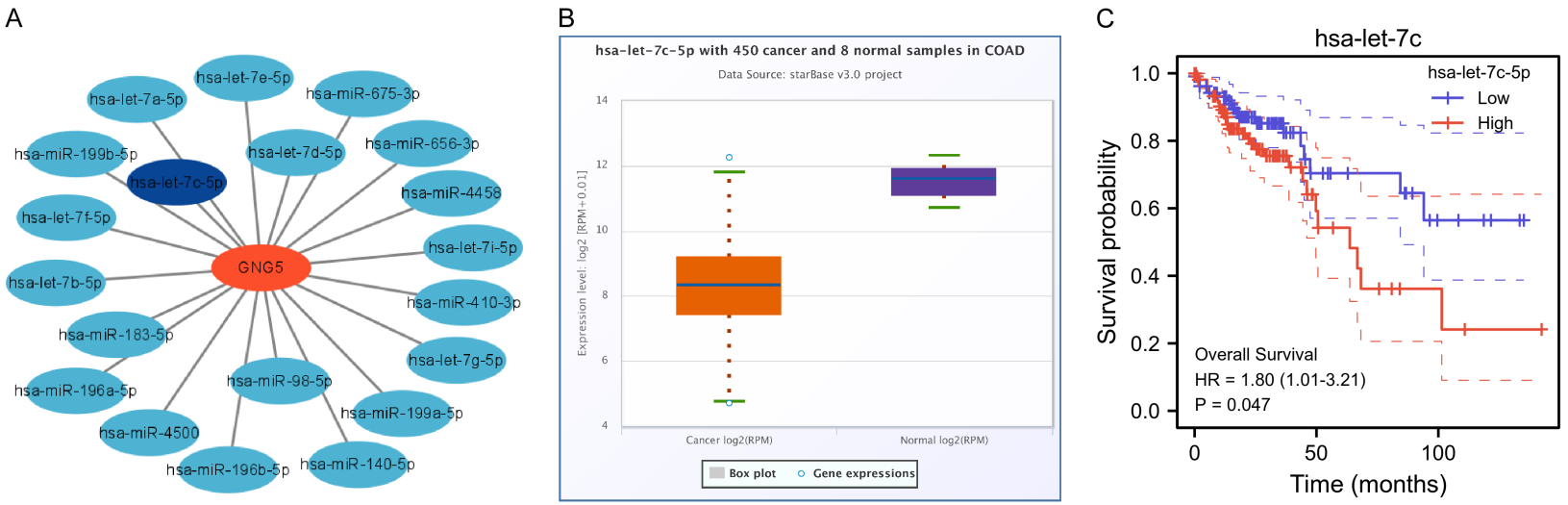

Figure 5. microRNA let-7c-5p established as an upstream miRNA of GNG5 in CRC. A. The miRNA-GNG5 regulatory network was constructed in Cytoscape software. B. The expression of let-7c-5p in CRC and control normal samples determined by starBase database. let-7c-5p was significantly downregulation in CRC. C. The prognostic value of let-7C-5P in CRC was evaluated by Xiantao Academic, and the prognosis of high and low expression groups was significant.

Table 1. The expression correlation between predicted mRNA, miRNAs, and LncRNA in CRC analyzed by starBase database

mRNA	miRNA	R value	*P* value
GNG5	hsa-let-7c-5p	-0.173	2.31e-4
LncRNA	miRNA	R value	*P* value
DRAIC	hsa-let-7c-5p	-0.107	2.36E-02
SNHG4	hsa-let-7c-5p	-0.1	3.45E-02
LncRNA	mRNA	R value	*P* value
SNHG4	GNG5	0.18	0.00012
DRAIC	GNG5	0.131	4.44e-3

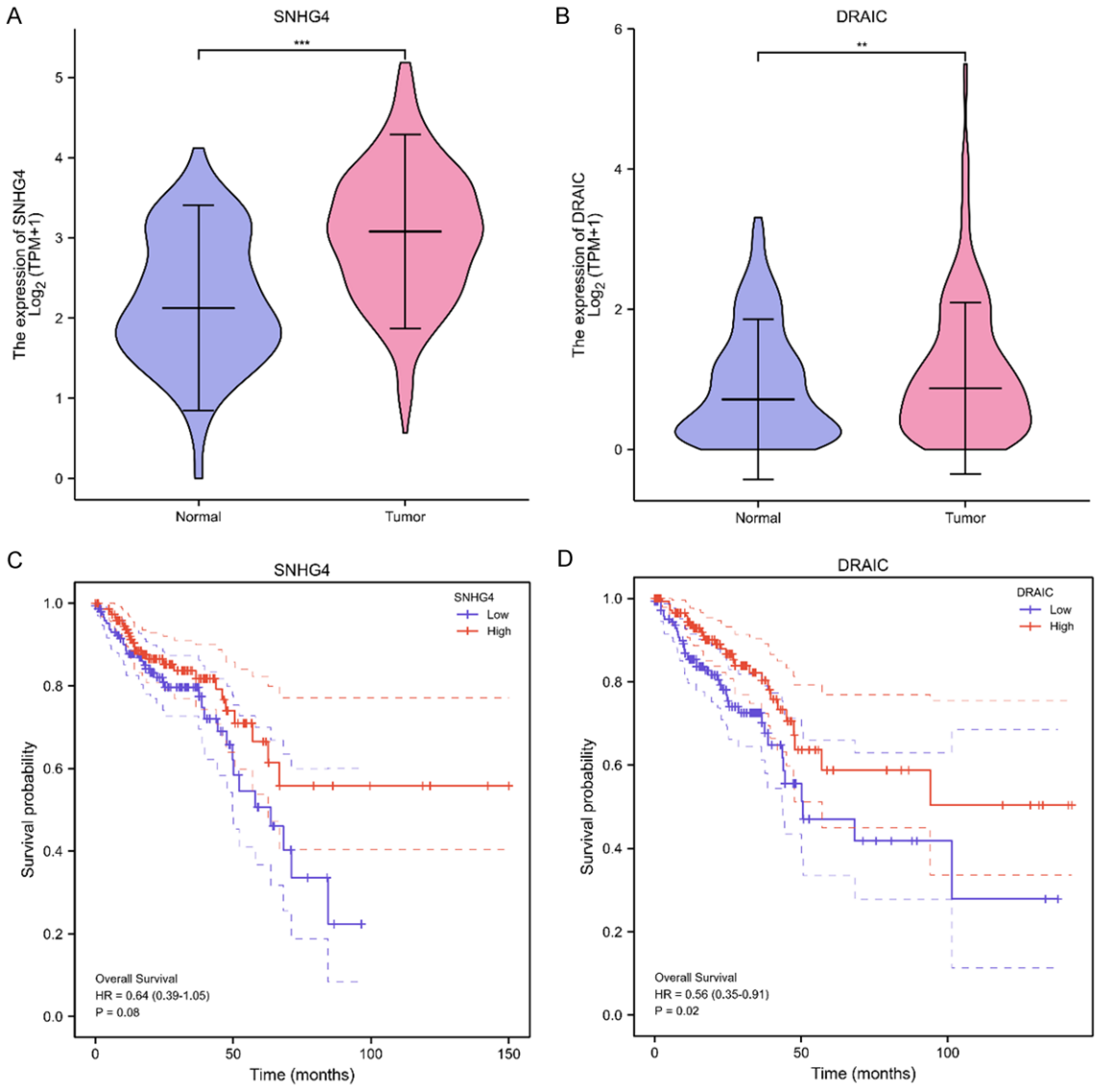

Figure 6. Expression analysis and survival analysis of let-7c-5p upstream lncRNA in CRC. (A, B) The expression of SNHG4 (A), DRAIC (B) in TCGA CRC compared with "TCGA normal" or "TCGA and GTEx normal" data. (C, D) The OS analysis for SNHG4 (C), DRAIC (D) in CRC.

immune escape. Considering their important oncogenic role, we were interested in understanding the relationship of GNG5 with PD1, PD-L1, or CTLA-4 in CRC. As suggested in

Table 2. Correlation analysis between GNG5 and immune cell biomarkers in CRC detected by GEPIA database

Immune cell	Biomarker	R value	*P* value
B cell	CD19	-0.15	0.012
	CD79A	-0.17	0.0039
CD8 T cell	CD8A	-0.11	0.062
	CD8B	-0.052	0.39
CD4 T cell	CD4	-0.15	0.011
M1 macrophage	NOS2	0.11	0.081
	IRF5	-0.11	0.066
	PTGS2	0.0068	0.91
M2 macrophage	CD163	-0.2	0.00089
	VSIG4	-0.17	0.0037
	MS4A4A	-0.2	0.00064
Neutrophil	CEACAM8	0.16	0.0096
	ITGAM	-0.2	0.00086
	CCR7	-0.12	0.055
Dentritic cell	HLA-DPB1	-0.11	0.075
	HLA-DQB1	-0.021	0.73
	HLA-DRA	-0.043	0.48
	HLA-DPA1	-0.067	0.27
	CD1C	-0.087	0.15
	NRP1	-0.17	0.0037
	ITGAX	-0.2	7E-04

Figure 8A, **8C**, **8E**, GNG5 expression was significantly negatively correlated with PD1 and CTLA-4 in CRC. This was adjusted by purity. We found a significant negative correlation of GNG5 with PD1 or CTLA-4 in CRC (**Figure 8B**, **8D**, **8F**). These findings are consistent with the results demonstrating that tumor immune escape can be involved in GNG5 mediated carcinogenesis of CRC.

Discussion

Colorectal cancer (CRC) is the third most common cancer and the fourth most common cause of cancer-related death due to distant metastasis. GNG5 is associated with a poor or better prognosis in a variety of human cancers, including CRC. Varying elucidated mechanisms in CRC carcinogenesis provide unexpected clues toward the prevention and promising prognostic biomarker and effective therapeutic target for CRC. There is increasing evidence that GNG5 plays a key role in the initiation and development of many human cancers, including CRC. The role of GNG5 in CRC carcinogenesis remains insufficient and needs more study. In this study, we used The Cancer Genome Atlas (TCGA) data to conduct pan-cancer analysis of GNG5 expression. This assisted in verifying GNG5 expression through GEPIA database. Our survival analysis for GNG5 in TCGA datasets indicated that CRC patients with high expression of GNG5 had a better prognosis. G-proteins are composed of three subunits α, β, and γ subunits, known as GTP binding proteins. These play essential roles of signal transduction in response to a wide variety of hormones and neurotransmitters that cross membranes and trigger physiological and biochemical reactions defined within the cells [26, 27]. G-proteins regulate the fundamental processes of life including cell metabolism, secretion, growth, proliferation, differentiation, pathological changes, and cell death [7]. GNG5 is a subunit of g protein that is involved in promoting the proliferation and migration of cancer cells [28]. It is explored more often in cancer therapies. GNG5 have recently emerged as key regulators of vasculogenesis, angiogenesis, and tumorigenesis. Our results showed GNG5 playing as an oncogenic role in CRC.

It has been reported that ncRNAs including miRNAs, lncRNAs and circRNAs are involved in regulating gene expression through the mechanism of ceRNA [29-33]. To explore upstream regulatory miRNAs of GNG5, we introduced two predictive programs, including miRmap and TargetScan, to predict miRNAs that bind to GNG5. We ended up with 20 miRNAs. Most of these miRNAs have been found in CRC as tumor suppressor miRNAs. For example, the increase of exosomal let-7c-5p extracted from the plasma of colorectal cancer patients was more significant when the prognostic indicators of cancer stage, recurrence, venous invasion, and lymphatic invasion were poor [34]. Through correlation analysis, expression analysis, and survival analysis, we selected let-7c-5p as the most promising upstream tumor suppressor miRNA of GNG5. Previous studies have shown that let-7c-5p plays an inhibitory role in regulating the proliferation and migration of CRC. The miRNAs regulating target gene expression based on endogenous RNA were negatively correlated with GNG5. The let-7C-5p expression was down-regulated in colon cancer. High let-7C-5p expression was negatively correlated with patient prognosis. All these findings sug-

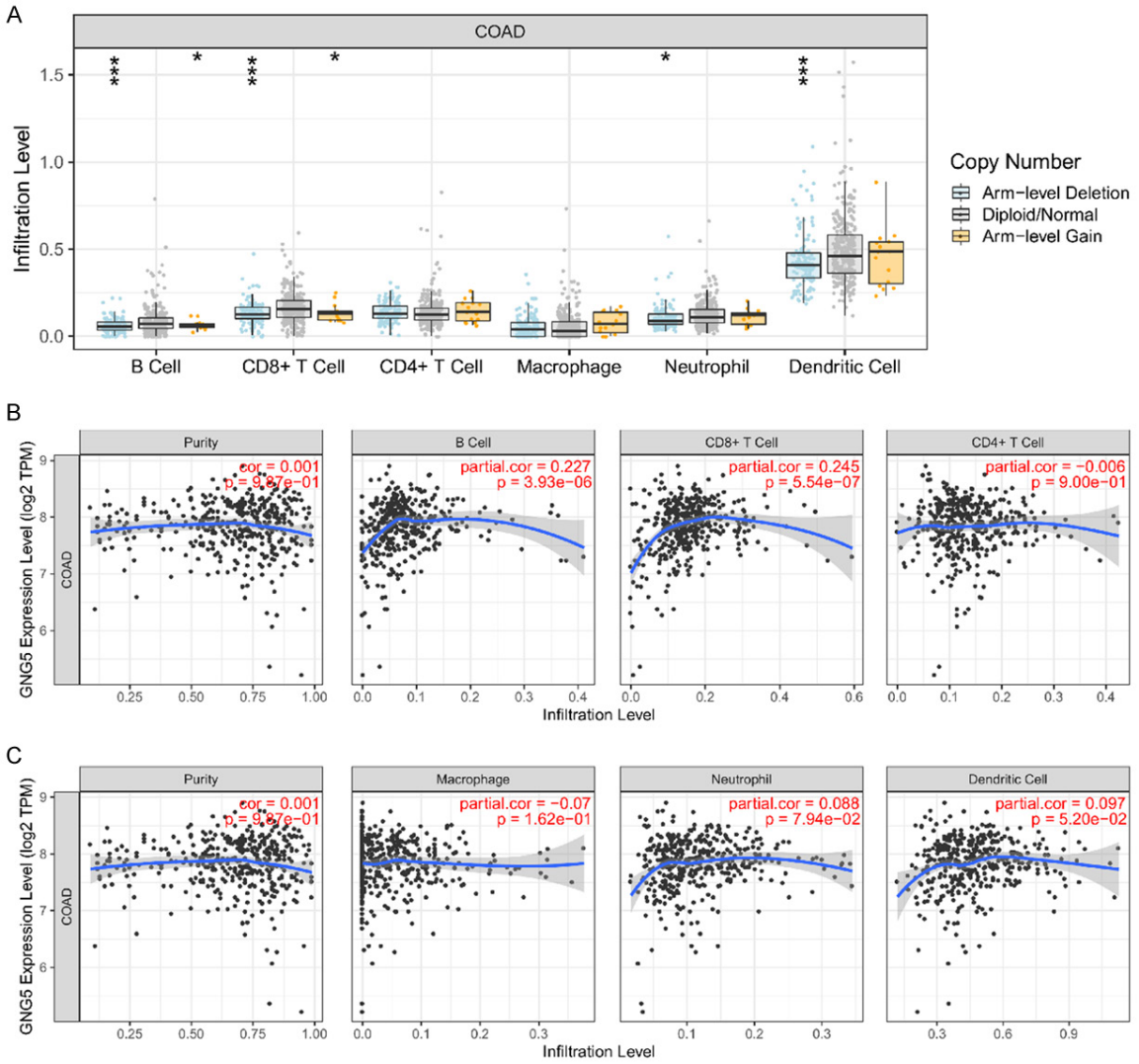

Figure 7. Relationship between immune cell infiltration and GNG5 level in colorectal cancer. A. Infiltration levels of different immune cells with different copy numbers of GNG5 in CRC. B. The correlation of GNG5 expression level with B cell, CD8+ T cell, CD4+ T cell infiltration level in CRC. C. The correlation of GNG5 expression level with macrophage, neutrophil, or dendriticcell infiltration level in CRC.

gested that let-7C-5P is the most potential miRNA regulating GNG5 in colon cancer.

The ceRNA hypothesis would imply the potential lncRNAs of let-7c-5p/GNG5 axis should be oncogenic lncRNAs in CRC [35]. We predicted upstream lncRNA of let-7c-5p/GNG5 axis and 53 possible lncRNAs were found. Through expression analysis, survival analysis and correlation analysis, SNHG4, and DRAIC were identified as the two most likely up-regulated lncRNAs. The lncRNA-SNHG4 has been reported to function as an oncogene in a variety of malignancies, including CRC. LncRNA-SNHG4 regulates colorectal cancer cell cycle and cell proliferation and upregulation of MET promote CRC cell proliferation, aggregation, formation, invasion, and immune escape of CRC cells, leading to the progression of CRC [36, 37]. DRAIC is a 1.7 kb long transcript comprising five exons. These were located in in the cytoplasm and have been shown to inhibit invasion. Low expression of DRAIC was associated with poor patient survival in prostate cancers [38]. Low levels of DRAIC are predictors of poor prognosis in several malignancies such as bladder cancer, hepatocellular carcinoma, glioma, lung adenocarcinoma, renal clear cell carcinoma,

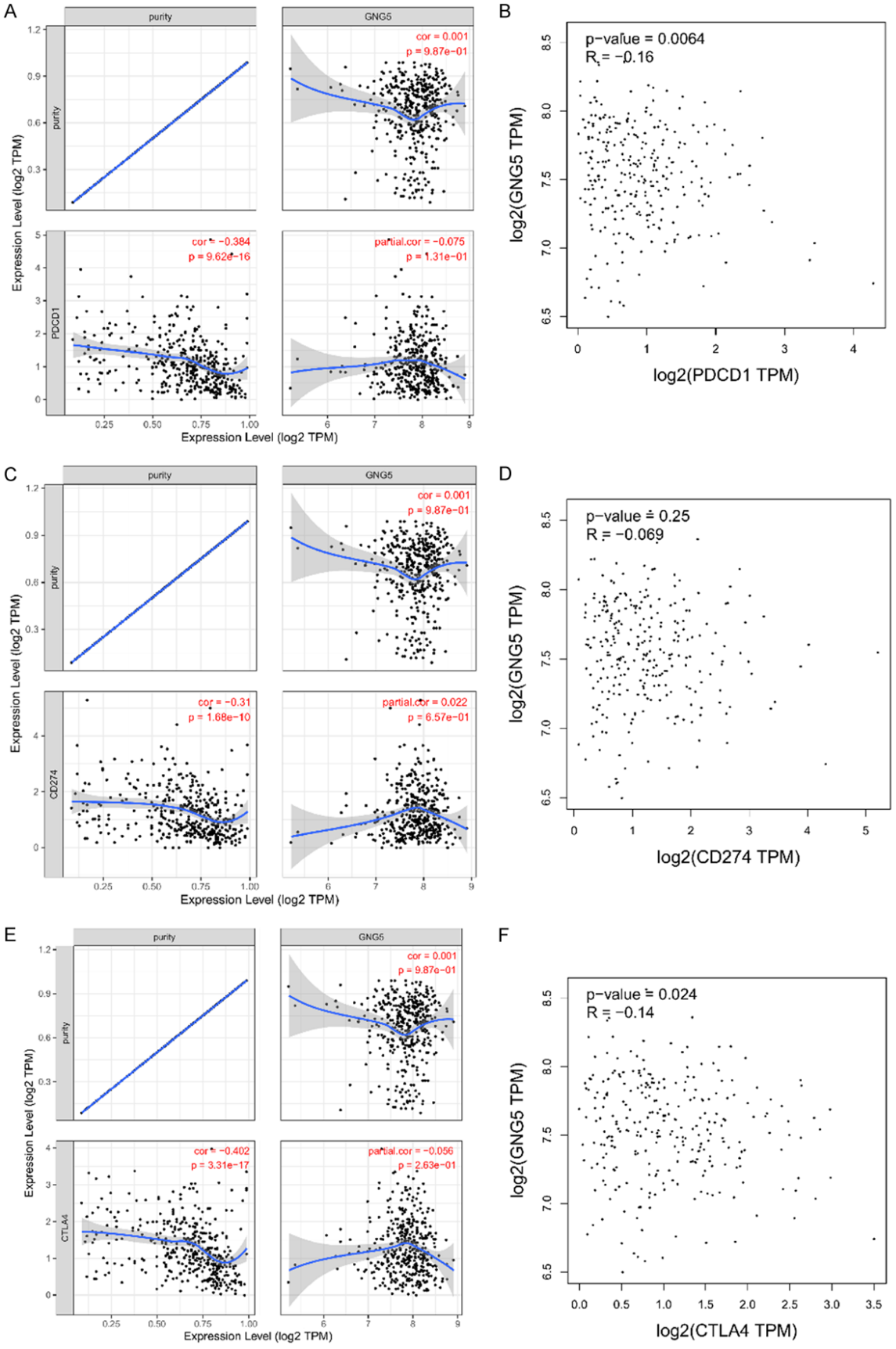
A
purity
GNG5
cor = 0.001
p = 9.87e−01
cor = −0.384
p = 9.62e−16
partial.cor = −0.075
p = 1.31e−01
PDCD1
Expression Level (log2 TPM)
B
p−value = 0.0064
R = −0.16
log2(GNG5 TPM)
log2(PDCD1 TPM)
C
cor = 0.001
p = 9.87e−01
cor = −0.31
p = 1.68e−10
partial.cor = 0.022
p = 6.57e−01
CD274
D
p−value = 0.25
R = −0.069
log2(CD274 TPM)
E
cor = 0.001
p = 9.87e−01
cor = −0.402
p = 3.31e−17
partial.cor = −0.056
p = 2.63e−01
CTLA4
F
p−value = 0.024
R = −0.14
log2(CTLA4 TPM)

Figure 8. Correlation between GNG5 expression and PD-1, PD-L1 and CTLA-4 expression in CRC. A. Correlation of the expression between GNG5 and PD-1 in CRC using TIMER. B. Spearman correlation of GNG5 with expression of PD-1 in CRC adjusted by purity using TIMER. C. Correlation of the expression between GNG5 and PD-L1 in CRC using TIMER. D. The expression correlation of GNG5 with PD-L1 in CRC adjusted by purity using TIMER. E. Correlation of the expression between GNG5 and CTLA-4 in CRC using TIMER. F. The expression correlation of GNG5 with CTLA-4 in CRC adjusted by purity using TIMER.

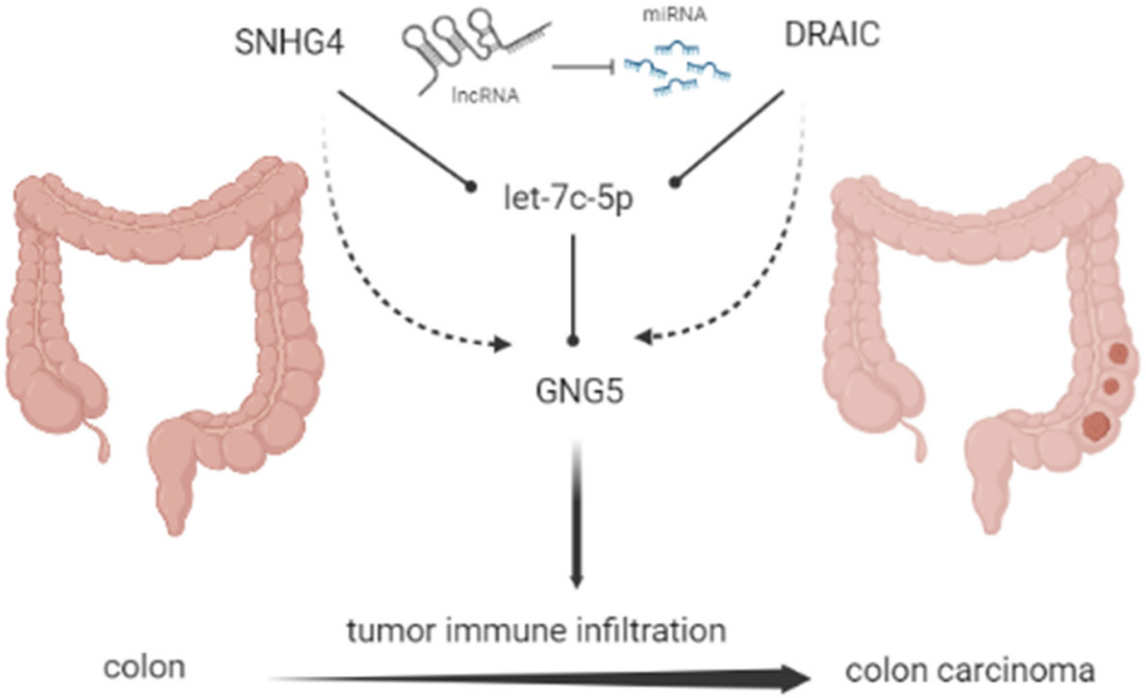

Figure 9. The model of SNHG4/DRAIC-let-7c-5p-GNG5 axis in carcinogenesis of CRC.

and gastric adenocarcinoma. The nuclear factor-κB (NF-κB) is a mastersignal transduction pathway regulating inflammation in different cancers including CRC [39]. The NF-κB pathway has been shown to stimulate soft agar colony formation in breast, melanoma, colon, and bone cancers. DRAIC inhibits cancer cell migration and invasion [38, 40]. These studies, taken together, SNHG4/DRAIC/let-7c-5p/GNG5 axis were identified as potential regulatory pathways in CRC.

Several studies have confirmed that tumor immune cell infiltration can affect the efficacy of chemotherapy, radiotherapy, or immunotherapy, and affect the treatment outcome of patients. Along these lines, our work suggests GNG5 correlated with immune cells, CD4+ T cell, macrophage, neutrophil, and dendritic cell were significantly negatively correlated in CRC. In the current study we explored, GNG5 was significantly positively correlated with these biomarkers infiltrating immune cells. Such an effect could partially account for GNG5-mediated oncogenic roles by tumor immune infiltration in CRC.

Optimal immunotherapy requires adequate infiltration of immune cells into the tumor microenvironment. It depends on the expression level of immune checkpoints [41]. In this study, we evaluated the relationship between GNG5 and immune checkpoints. The results of this study demonstrate the high expression of GNG5 have been strongly linked to PD1, CTLA-4 in CRC. Our study suggested that GNG5 reduced the efficacy of immunotherapy for colorectal cancer.

Conclusions

GNG5 was highly expressed in diverse cancer types and showed a positive association with better prognosis in CRC. Targeting endothelial

cells and different members of the GNG5 family of tumor cells is a promising therapeutic approach to prevent tumor angiogenesis, growth, and metastasis. Based on our results, we analyzed and identified the upstream regulation mechanism of GNG5 associated with SNHG4/DraIC-let-7C-5P axis in CRC. We believe that SNHG4/DrAIC-let-7C-5P-GNG5 axis play an important role in the occurrence and development of colon cancer (**Figure 9**). Our current findings support that finding and indicate that GNG5 lead to oncogenic through reducing expression of immune checkpoint and immune cell infiltration. These results provided a constructive groundwork for the assessment of immunetherapies in future clinical trials of CRC. These clinical uses need to be validated by large clinical trials.

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Disclosure of conflict of interest

None.

Authors' contributions

Bo Zhao and Yongjun Chen contributed equally to this study. Bo Zhao contributed to this study, analyzed the data, and wrote the manuscript. Yongjun Chen designed the experiments. Wenqi Lu and Wenjin Chen checked and revised the manuscript and confirmed all the data in the manuscript. Xiaoyong Cai performed the experiments for the revised manuscript. All authors read and approved the final manuscript.

Abbreviations

CRC, Colorectal cancer; TCGA, The Cancer Genome Atlas; GTEx, The Genotype-Tissue Expression; GNG5, G protein subunit gamma 5; ncRNAs, Noncoding RNAs; lncRNAs, long noncoding RNAs; GPCR, G protein-coupled receptor; TGF-β, transforming growth factor beta; miRNAs, microRNAs; HPA, the Human Protein Atlas; IHC, immunohistochemistry; OS, overall survival; RFS, The disease-free survival; NF-κb, nuclear factor-κB.

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References

[1] Ghatak S, Satapathy SR and Sjölander A. DNA methylation and gene expression of the cysteinyl leukotriene receptors as a prognostic and metastatic factor for colorectal cancer patients. Int J Mol Sci 2023; 24: 3409.

[2] Wasan HS, Gibbs P, Sharma NK, Taieb J, Heinemann V, Ricke J, Peeters M, Findlay M, Weaver A, Mills J, Wilson C, Adams R, Francis A, Moschandreas J, Virdee PS, Dutton P, Love S, Gebski V and Gray A; FOXFIRE trial investigators; SIRFLOX trial investigators; FOXFIRE-Global trial investigators; van Hazel G and Sharma RA. First-line selective internal radiotherapy plus chemotherapy versus chemotherapy alone in patients with liver metastases from colorectal cancer (FOXFIRE, SIRFLOX, and FOXFIRE-Global): a combined analysis of three multicentre, randomised, phase 3 trials. Lancet Oncol 2017; 18: 1159-1171.

[3] Li T, Yu J, Chen Y, Liu R, Li Y, Wang YX, Wang JJ and Zhu P. Preventive intraperitoneal hyperthermic perfusion chemotherapy for patients with T4 stage colon adenocarcinoma. Tech Coloproctol 2021; 25: 683-691.

[4] Ivanova M, Venetis K, Guerini-Rocco E, Bottiglieri L, Mastropasqua MG, Garrone O, Fusco N and Ghidini M. HER2 in metastatic colorectal cancer: pathology, somatic alterations, and perspectives for novel therapeutic schemes. Life (Basel) 2022; 12: 1403.

[5] Guler I, Askan G, Klostergaard J and Sahin IH. Precision medicine for metastatic colorectal cancer: an evolving era. Expert Rev Gastroenterol Hepatol 2019; 13: 919-931.

[6] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.

[7] Syrovatkina V, Alegre KO, Dey R and Huang XY. Regulation, signaling, and physiological functions of G-proteins. J Mol Biol 2016; 428: 3850-3868.

[8] Ohta M, Mimori K, Fukuyoshi Y, Kita Y, Motoyama K, Yamashita K, Ishii H, Inoue H and Mori M. Clinical significance of the reduced expression of G protein gamma 7 (GNG7) in oesophageal cancer. Br J Cancer 2008; 98: 410-417.

[9] Xu S, Zhang H, Liu T, Chen Y, He D and Li L. G protein γ subunit 7 loss contributes to progression of clear cell renal cell carcinoma. J Cell Physiol 2019; 234: 20002-20012.

[10] Ding J, Li Y, Fan H, Xu W, Gao R, Bai S, Zhu Z, Yang W, Gong Y, Yang J and Zhou J. Knockdown of PSMC3IP suppresses the proliferation and xenografted tumorigenesis of hepatocellular carcinoma cell. J Cell Biochem 2019; 120: 5449-5458.
[11] Chen L, Lu D, Sun K, Xu Y, Hu P, Li X and Xu F. Identification of biomarkers associated with diagnosis and prognosis of colorectal cancer patients based on integrated bioinformatics analysis. Gene 2019; 692: 119-125.
[12] Miwa T, Kanda M, Tanaka H, Tanaka C, Kobayashi D, Umeda S, Iwata N, Hayashi M, Yamada S, Fujii T, Fujiwara M and Kodera Y. FBXO50 enhances the malignant behavior of gastric cancer cells. Ann Surg Oncol 2017; 24: 3771-3779.
[13] Ahmad W, Li S, Chen H, Tuck-Muller CM, Pittler SJ and Aronson NN Jr. Lysosomal chitobiase (CTB) and the G-protein γ5 subunit (GNG5) genes co-localize to human chromosome 1p22. Cytogenet Cell Genet 1995; 71: 44-46.
[14] Hua X, Chen J and Wu L. Identification of candidate biomarkers associated with apoptosis in melanosis coli: GNG5, LPAR3, MAPK8, and PSMC6. Biosci Rep 2019; 39: BSR20181369.
[15] Brooks C, Murphy J, Belcastro M, Heller D, Kolandaivelu S, Kisselev O and Sokolov M. Farnesylation of the Transducin G protein gamma subunit is a prerequisite for its ciliary targeting in rod photoreceptors. Front Mol Neurosci 2018; 11: 16.
[16] Bill MA, Bakan C, Benson DM Jr, Fuchs J, Young G and Lesinski GB. Curcumin induces proapoptotic effects against human melanoma cells and modulates the cellular response to immunotherapeutic cytokines. Mol Cancer Ther 2009; 8: 2726-2735.
[17] Canovas B and Nebreda AR. Diversity and versatility of p38 kinase signalling in health and disease. Nat Rev Mol Cell Biol 2021; 22: 346-366.
[18] Alsaleem M, Toss MS, Joseph C, Aleskandarany M, Kurozumi S, Alshankyty I, Ogden A, Rida PCG, Ellis IO, Aneja R, Green AR, Mongan NP and Rakha EA. The molecular mechanisms underlying reduced E-cadherin expression in invasive ductal carcinoma of the breast: high throughput analysis of large cohorts. Mod Pathol 2019; 32: 967-976.
[19] Karimi E, Azari H, Yari M, Tahmasebi A, Hassani Azad M and Mousavi P. Interplay between SARS-CoV-2-derived miRNAs, immune system, vitamin D pathway and respiratory system. J Cell Mol Med 2021; 25: 7825-7839.
[20] Consortium U. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res 2019; 47: D506-D515.
[21] Wang JQ, Liu YR, Xia Q, Chen RN, Liang J, Xia QR and Li J. Emerging roles for NLRC5 in immune diseases. Front Pharmacol 2019; 10: 1352.
[22] Smyth GK, Michaud J and Scott HS. Use of within-array replicate spots for assessing differential expression in microarray experiments. Bioinformatics 2005; 21: 2067-2075.
[23] Li JH, Liu S, Zhou H, Qu LH and Yang JH. starBase v2. 0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res 2014; 42: D92-D97.
[24] Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017; 45: W98-W102.
[25] Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, Li B and Liu XS. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res 2017; 77: e108-e110.
[26] Ilyaskina OS, Lemoine H and Bünemann M. Lifetime of muscarinic receptor-G-protein complexes determines coupling efficiency and G-protein subtype selectivity. Proc Natl Acad Sci U S A 2018; 115: 5016-5021.
[27] Wingler LM and Lefkowitz RJ. Conformational basis of G protein-coupled receptor signaling versatility. Trends Cell Biol 2020; 30: 736-747.
[28] Yuan Z, Ren R and Xu Z. G protein subunit gamma 5 promotes the proliferation, metastasis and glycolysis of breast cancer cells through the Wnt/β-catenin pathway. Anticancer Drugs 2022; 33: 1004-1011.
[29] Lou W, Ding B, Wang J and Xu Y. The involvement of the hsa_circ_0088494-miR-876-3p-CTNNB1/CCND1 axis in carcinogenesis and progression of papillary thyroid carcinoma. Front Cell Dev Biol 2020; 8: 605940.
[30] Gao S, Ding B and Lou W. microRNA-dependent modulation of genes contributes to ESR1's effect on ERα positive breast cancer. Front Oncol 2020; 10: 753.
[31] Ghafouri-Fard S, Shoorei H, Anamag FT and Taheri M. The role of non-coding RNAs in controlling cell cycle related proteins in cancer cells. Front Oncol 2020; 10: 608975.
[32] Razavi ZS, Tajiknia V, Majidi S, Ghandali M, Mirzaei HR, Rahimian N, Hamblin MR and Mirzaei H. Gynecologic cancers and non-coding RNAs: epigenetic regulators with emerging roles. Crit Rev Oncol Hematol 2021; 157: 103192.
[33] Fabrizio FP, Sparaneo A and Muscarella LA. NRF2 regulation by noncoding RNAs in cancers: the present knowledge and the way forward. Cancers (Basel) 2020; 12: 3621.
[34] Cho WC, Kim M, Park JW, Jeong SY and Ku JL. Exosomal miR-193a and let-7g accelerate cancer progression on primary colorectal cancer and paired peritoneal metastatic cancer. Transl Oncol 2021; 14: 101000.

[35] Salmena L, Poliseno L, Tay Y, Kats L and Pandolfi PP. A ceRNA hypothesis: the Rosetta stone of a hidden RNA language? Cell 2011; 146: 353-358.
[36] Zhou Z, Tan F, Pei Q, Li C, Zhou Y, Li Y and Pei H. lncRNA SNHG4 modulates colorectal cancer cell cycle and cell proliferation through regulating miR-590-3p/CDK1 axis. Aging (Albany NY) 2021; 13: 9838-9858.
[37] Zhou N, Chen Y, Yang L, Xu T, Wang F, Chen L, Liu J and Liu G. LncRNA SNHG4 promotes malignant biological behaviors and immune escape of colorectal cancer cells by regulating the miR-144-3p/MET axis. Am J Transl Res 2021; 13: 11144.
[38] Sakurai K, Reon BJ, Anaya J and Dutta A. The lncRNA DRAIC/PCAT29 locus constitutes a tumor-suppressive nexus. Mol Cancer Res 2015; 13: 828-838.
[39] Mokashi CS, Schipper DL, Qasaimeh MA and Lee REC. A system for analog control of cell culture dynamics to reveal capabilities of signaling networks. iScience 2019; 19: 586-596.
[40] Zhang Z, Hu X, Kuang J, Liao J and Yuan Q. LncRNA DRAIC inhibits proliferation and metastasis of gastric cancer cells through interfering with NFRKB deubiquitination mediated by UCHL5. Cell Mol Biol Lett 2020; 25: 29.
[41] Chae YK, Arya A, Iams W, Cruz MR, Chandra S, Choi J and Giles F. Current landscape and future of dual anti-CTLA4 and PD-1/PD-L1 blockade immunotherapy in cancer; lessons learned from clinical trials with melanoma and non-small cell lung cancer (NSCLC). J Immunother Cancer 2018; 6: 39.