

## Original Article

# Over-expression of long non-coding RNA GAPLINC promotes colorectal cancer cell metastasis and poor prognosis

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**Abstract:** Objective: Long non-coding RNAs (lncRNAs) play critical regulatory roles in cancer progression. However, the roles of lncRNAs in colorectal cancer (CRC) are not yet well elucidated. The aim of the present study was to assess the potential role of lncRNA GAPLINC in the pathogenesis of CRC. Method: GAPLINC expression in CRC tissues and adjacent non-tumor tissues were collected from 64 patients and measured by quantitative real-time PCR (qRT-PCR). GAPLINC correlation with clinicopathological features was also analyzed. In addition, the biological roles of GAPLINC were evaluated by MTT assay, migration assay and invasion assay. Results: qRT-PCR data showed that GAPLINC was elevated in CRC tissues and cell lines compared with adjacent non-tumor tissues and normal human intestinal epithelial cell line. Moreover, high expression of GAPLINC was correlated with invasion, lymph node metastases, and TNM stage in CRC patients. In addition, our in vitro results showed that knockdown expression of GAPLINC could suppress the proliferation, migration and invasion capacity of CRC cells. Conclusions: Our findings suggested that GAPLINC play a vital role in CRC proliferation and metastasis and could represent a potential prognostic biomarker and therapeutic target in CRC patients.

**Keywords:** Colorectal cancer, long non-coding RNAs, GAPLINC, quantitative real-time PCR

## Introduction

Colorectal carcinoma (CRC) is one of the leading causes of cancer related death worldwide [1]. The CRC incidence and mortality in China increase rapidly in the past several decades [2]. Although many identified molecules play roles in the way CRC progression and metastasis, the mechanisms of CRC are still unclear [3]. For accurate diagnosis and adequate treatment of CRC, identification and understanding of the molecules responsible for cancer progression are urgent [4].

Recent integrative genomic studies have revealed that the human genome encodes more than 10,000 long non-coding RNAs (lncRNAs) with limited or no protein-coding capacity [5]. Although a small number of lncRNAs have been functionally characterized, a large number of members in the class remain functionally uncharacterized [6]. Growing evidence suggested that cancer lncRNAs may mediate oncogenic or tumor-suppressing

effects and may be a new class of cancer biomarkers and therapeutic targets [7]. For example, Wang et al. showed that lncRNA HOTAIR acted as a tumor oncogene to promote tumor growth and metastasis in human osteosarcoma [8]. Hu et al. suggested that lncRNA MALAT1 served as an oncogene in esophageal squamous cell carcinoma (ESCC) and regulated ESCC growth by modifying the ATM-CHK2 pathway [9]. Wang et al. reported that lncRNA MEG3 acts as novel suppressor of migration and invasion by targeting Rac1 gene in thyroid carcinoma progression [10]. Hu et al. reported that GAS5 acted as a tumor suppressor in hepatocellular carcinoma cells through negative regulation of miR-21 and its targets and proteins about migration and invasion in cancer cells [11].

However, our current knowledge about the expression patterns and functional roles of lncRNAs is still limited. In previous study, Hu et al. revealed that the expression of GAPLINC was correlated with gastric cancer [12]. To our

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**Table 1.** Correlation between GAPLINC expression and clinicopathological features of CRC

Parameters	Group	Total	lncRNA GAPLINC expression		P value
			High	Low	
Gender	Male	38	21	17	0.309
	Female	26	11	15	
Age (years)	<60	30	14	16	0.616
	≥60	34	18	16	
Tumor size (cm)	<5 cm	33	18	15	0.453
	≥5 cm	31	14	17	
Histological grade	Well and moderately	43	18	25	0.062
	Poorly	21	14	7	
Local invasion	T1-T2	22	7	15	0.035
	T3-T4	42	25	17	
Lymph nodes metastasis	Negative	46	18	28	0.005
	Positive	18	14	4	
TNM stage	I-II	27	9	18	0.023
	III-IV	37	23	14	

knowledge, no more evidences proved that GAPLINC was correlated with other carcinomas. Thus, in this study, we determined the expression patterns of GAPLINC in CRC tissues and paired non-tumor tissues. Moreover, siRNA-mediated silence was also performed to assess the impact of GAPLINC on CRC cell proliferation, migration and invasion ability in vitro.

### Materials and methods

#### Patients and tissue samples

A total of 64 CRC tissues and paired adjacent non-tumor tissues were obtained from patients who had undergone surgical resection of colorectal cancer between 2007 and 2008 at the Qilu Hospital of Shandong University, China. The CRC diagnosis was confirmed by an experienced pathologist. All of the tissue samples were washed with sterile phosphate-buffered saline before being snap frozen in liquid nitrogen and stored at -80°C until total RNA was extracted. No patients had been treated with radiotherapy or chemotherapy before surgery. This study was approved by the Ethics Committee of Shandong University and informed consent was obtained from each patient involved in the study.

#### Cell lines and transfection

Cell lines HCT8, HCT116, HT29 (human colon cancer cell lines) and FHC (normal human intestinal epithelial cell line) were obtained from

Shanghai Cell Collection, Chinese Academy of Sciences. All above cell lines were maintained in DMEM (Hyclone) containing 10% fetal bovine serum (FBS, Hyclone) and cultured at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>.

HCT116 cells were transfected with either 50 nM siRNA targeting GAPLINC (si-GAPLINC) or scrambled negative controls (si-NC) (GenePharma) using the Lipofectamine 2000 transfection reagent (Invitrogen) according to the instructions. The target sequence for GAPLINC siRNAs were

5'-AUAGGUCAUAGCAUCCAAUUGC-3' (si-GAPLINC). After 48 h, the efficiency of GAPLINC knockdown was confirmed via qRT-PCR.

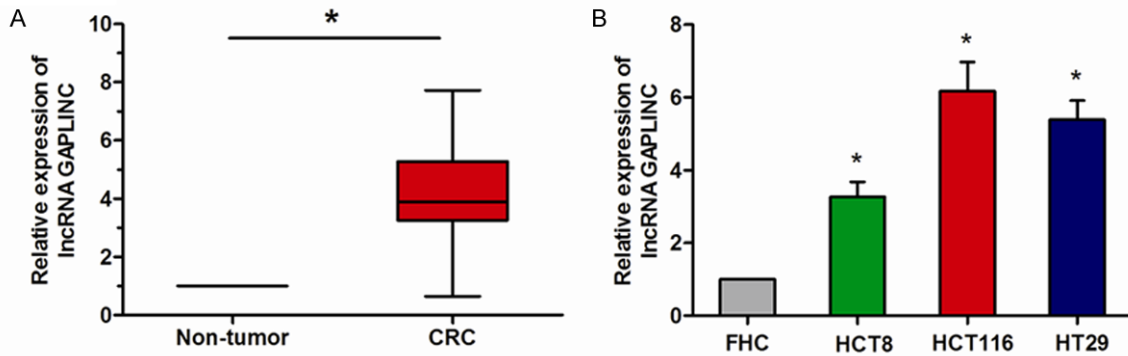
#### RNA isolation, reverse transcription, and qRT-PCR

Total RNA was extracted from tissues and cell lines using the Trizol reagent (Invitrogen) following the manufacturer's protocol. RNA was reverse transcribed into cDNAs using the Prime-Script™ one step RT-PCR kit (Takara). GAPLINC expression level was determined by qRT-PCR using the specific primers according Hu's report [12]. GAPDH was used as an internal control. The 2<sup>-ΔΔCt</sup> method was performed to calculate the relative amount of GAPLINC compared with GAPDH expression. qRT-PCR reactions were performed using the ABI7500 System (Applied Biosystems) and SYBR Green PCR Master Mix (Takara). Each experiment was performed in triplicate.

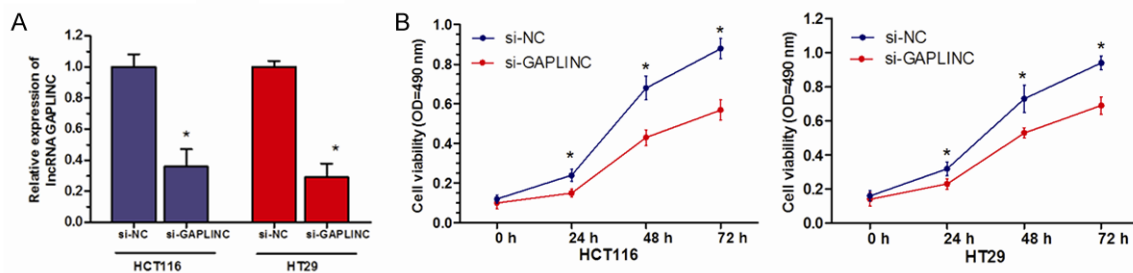
#### Cell proliferation assay

The transfected cell lines were subsequently harvested for cell proliferation assay (MTT assay) following the manufacturer's protocol. In briefly, the transfected cells were plated in flat bottom 96-well plates supplemented with 100 μl DMEM per well. After incubation for 24, 48, and 72 hours respectively, 10 μl of MTT was added to each well, then the medium was removed after 4 hours of culture and subse-

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**Figure 1.** Relative expression levels of GAPLINC in 64 paired CRC and adjacent non-tumor tissues (A) and in three CRC cell lines (B). The expression levels were determined using a qRT-PCR assay, and GAPDH was used as an internal control. \*P<0.05.



**Figure 2.** Inhibition of GAPLINC suppressed proliferation of CRC cells. A. qRT-PCR revealed that GAPLINC was efficiently knocked down by treatment with si-GAPLINC in HCT116 and HT29 cells. B. MTT assay showed that si-GAPLINC significantly inhibited cell proliferation in HCT116 and HT29 cells. \*P<0.05.

quently supplemented with 150 ml DMSO per well. Colorimetric analysis was performed on a microplate reader at 490 nm wavelength. The results represent the average of three replicates under the same conditions.

### Cell migration and invasion assays

For migration assay, the transfected cells were seeded into the upper chamber of transwell plates in a 24-well format with 8 mm diameters (Corning Costar). 600  $\mu$ l of DMEM containing 5% FBS were added to the bottom chamber as a chemo attractant. After 24 hours of culture, cells were fixed with methanol and were stained with crystal violet. Remaining cells were removed from the top of the permeable membrane using a cotton swab. Then cells that migrated through the upper chamber were counted in five random fields under a light microscope, and the average value of five fields was expressed.

For invasion assay, the top chambers were coated with basement membrane Matrigel (Becton-Dickinson) at 37°C for 30 min.

Transfected cells with serum-free DMEM were added into the top chambers, the bottom chambers were filled with DMEM containing 10% FBS. After 24 hours of incubation, the cells that invaded the reverse side of top chambers were fixed, stained, and calculated using a light microscope. Each invasion assay was performed in three replicates.

### Statistical analysis

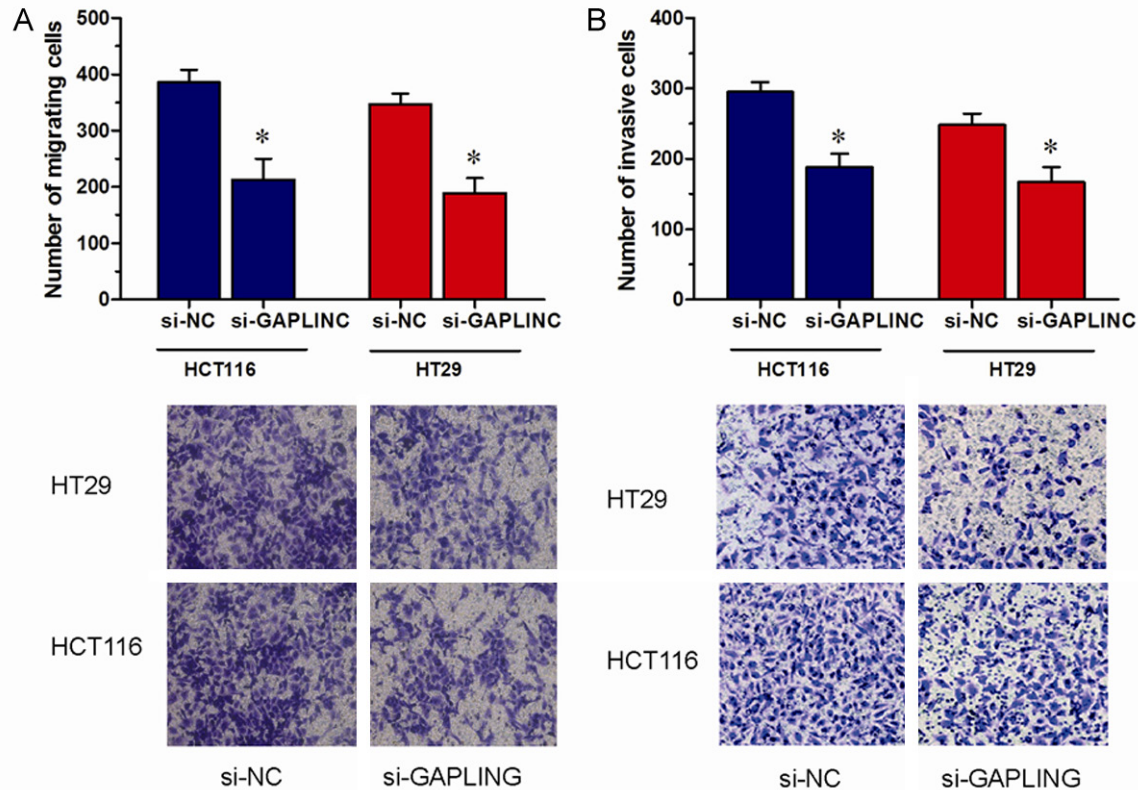
Statistical analyses were performed using SPSS version 18.0 software. Comparison of continuous data was analyzed using an independent t-test between the two groups. Categorical data were analyzed using the chi-square test. P values less than 0.05 were considered statistically significant.

## Results

### Correlation between lncRNA GAPLINC expression and clinicopathologic features

The expression of GAPLINC in 64 paired CRC tissues and adjacent non-tumor tissues from

## LncRNA GAPLINC expression in CRC



**Figure 3.** Silence of GAPLINC inhibited migration and invasion of CRC cells. A. Cell migration assay showed that si-GAPLINC significantly inhibited cell migration ability in HCT116 and HT29 cells. B. Cell invasion assay showed that si-GAPLINC significantly inhibited cell invasion ability in HCT116 and HT29 cells. \* $P < 0.05$ .

CRC patients were detected by qRT-PCR. Compared with the levels of the adjacent non-tumor tissues, a significant up-regulated of GAPLINC was observed in CRC patients ( $P < 0.05$ , **Figure 1A**). Furthermore, CRC cell lines were also tested, compared with normal human intestinal epithelial cell line FHC, GAPLINC expression level was also increased in all three CRC cell lines (HCT8, HCT116 and HT29) ( $P < 0.05$ , **Figure 1B**). To explore the association between GAPLINC and clinicopathological features, the 64 CRC patients were divided into two groups based on the median value of relative GAPLINC expression. As shown in **Table 1**, GAPLINC expression was correlated with invasion, lymph node metastases, and TNM stage ( $P < 0.05$ ). However, there were no significant correlations between GAPLINC expression and other clinicopathologic features including gender, age, tumor size, and histological grade ( $P > 0.05$ ). Taken together, these observations indicated that over-expression of GAPLINC might have important roles in CRC progression and metastasis.

### *Knockdown of GAPLINC expression decreases CRC cell proliferation*

To investigate the biological function of GAPLINC in tumor progression, we designed siRNAs to inhibit GAPLINC in vitro. At 48 hours after treatment, qRT-PCR showed that expression of GAPLINC in CRC cells was effectively knocked down ( $P < 0.05$ , **Figure 2A**). MTT assay demonstrated that down-regulated expression of GAPLINC significantly inhibited cell proliferation of HCT116 and HT29 cells compared with the scrambled negative control ( $P < 0.05$ , **Figure 2B**). Thus, our findings indicated that decreased expression of GAPLINC could effectively inhibit CRC cell proliferation in vitro.

### *Knockdown of GAPLINC expression inhibits CRC cell migration and invasion*

Transwell migration and invasion assays were performed to investigate the role of GAPLINC in the metastasis of CRC cells. Transwell migration assay showed that decreased expression



of GAPLINC dramatically suppressed cell migration in HCT116 and HT29 cells compared with the scrambled negative control ( $P < 0.05$ , **Figure 3A**). Similarly, transwell invasion assay demonstrated that knockdown of GAPLINC expression significantly inhibited the invasive capacity of HCT116 and HT29 cells ( $P < 0.05$ , **Figure 3B**). These findings suggested that decreased expression of GAPLINC could suppress the migration and invasion ability of CRC cells.

### Discussion

LncRNAs are non-coding RNAs greater than 200 nucleotides in length and can regulate gene expression in different biological processes transcriptionally or post-transcriptionally [13]. Previous studies indicated that lncRNAs are involved in a large number of biological processes, such as chromosome imprinting, histone modification, cell cycle regulation, cytoplasmic transport, and cell differentiation [14-16].

CRC is a kind of tumor including complex dynamic biological processes, and it initiates from multiple genetic and epigenetic alterations [17]. The functional role of lncRNAs in the occurrence and progression of CRC has got our attention. Up to now, several CRC associated lncRNA have been characterized. For example, Shi et al. showed that lncRNA RP11-462C24.1 was decreased expression in CRC and correlated with metastasis, furthermore, they found that RP11-462C24.1 could be a potential novel prognostic marker for CRC [18]. Han et al. reported that lncRNA UCA1 was up-regulated in CRC and regulated CRC cell proliferation, apoptosis and cell cycle distribution in CRC progression [19]. Ma et al. revealed that lncRNA CCAL could regulated colorectal cancer progression by activating Wnt/ $\beta$ -catenin signalling pathway via suppression of activator protein 2 $\alpha$  [20]. Those findings indicated that lncRNA play critical roles in CRC development and progression. However, little is known about the role of GAPLINC in CRC.

In this study, we explored the lncRNA GAPLINC expression level in 64 human CRC tissues. We found that GAPLINC was up-regulated in CRC tissues compared with the adjacent non-tumor tissues. Furthermore, our results showed that the relative expression level of GAPLINC was associated with invasion, lymph node metastasis,

and TNM stage, suggesting that over-expression of GAPLINC play important roles in CRC progression and metastasis. In vitro assay, our data revealed that knockdown of GAPLINC expression could inhibit CRC cell proliferation, migration and invasion, indicating that high expression of GAPLINC could promote the malignant phenotypes of CRC cells. Our data further supported the importance of GAPLINC in cellular biology and oncogenesis of CRC cells and indicated that GAPLINC was involved in functionally important elements in the development and progression of CRC.

In conclusion, we demonstrated that GAPLINC was up-regulated in CRC tissues and associated with advanced clinical features. Moreover, decreased expression of GAPLINC could inhibit CRC cell proliferation and metastasis in vitro. Thus, our findings indicated that GAPLINC could server as a potential therapeutic target of CRC.

### Disclosure of conflict of interest

None.

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