Letter to the Editor

rs3735664 Polymorphism Affecting *ELFN1-AS1* Adsorption on miR-1231 is Associated with Colorectal Cancer Susceptibility and Tumor Stage*

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Colorectal cancer (CRC) is a common and deadly disease, with over two million new cases and one million deaths in 2020[1]. Risk factors include lack of exercise, obesity, high red meat consumption, low intake of fiber, smoking, and alcohol consumption, as well as genetic factors[2,3]. *ELFN1-AS1*, located on chromosome 7p22.3, expresses a pro-oncogenic lncRNA[4-7]. Wang et al. reported that exosomal *ELFN1-AS1* from osteosarcoma cells could mediate macrophage M2 polarization by sponging miR-138-5p and miR-1291 to promote osteosarcoma tumorigenesis[4]. Lei et al. found that *ELFN1-AS1* could promote CRC proliferation and migration by modulating the miR-4644/TRIM44 axis[5]. Zhang et al. confirmed that *ELFN1-AS1* could facilitate the progression of esophageal cancer by promoting GFPT1 expression via sponging miR-183-3p[6]. Jie et al. found that *ELFN1-AS1* could accelerate the proliferation, invasion, and migration of ovarian cancer cells by directly interacting with miR-497-3p and then regulating CLDN4 expression[7]. These results suggest that *ELFN1-AS1* promotes the progression of various cancers, including CRC, osteosarcoma, esophageal cancer, and ovarian cancer, by acting as a competing endogenous RNA (ceRNA) and modulating miRNA expression. Bioinformatics analysis shows that a single nucleotide polymorphism (rs3735664 G>A) is located at the binding site of *ELFN1-AS1* to miR-599 and miR-1231 (Supplementary Figure S1 available in www.besjournal.com)[8]. MiR-599 and miR-1231 could be adsorbed by some sponges, which in turn promotes the progression of CRC[9,10]. Therefore, the rs3735664 polymorphism may be involved in the development and progression of CRC by affecting the ceRNA function of *ELFN1-AS1*.

In this study, 1,000 individuals of Chinese Han ethnicity were recruited, 500 of whom were diagnosed with sporadic CRC, confirmed by pathology. The other 500 were cancer-free individuals who had no family history of cancer, had no intestinal disease, and were enrolled in hospital physical examinations (Supplementary Table S1 available in www.besjournal.com). Patients with other digestive diseases were excluded. The average age of patients and cancer-free individuals was 59.18 ± 7.46 and 59.70 ± 7.63, respectively. Male participants made up the majority, with 274 (54.8%) of the CRC patients and 283 (56.6%) of the cancer-free individuals being male. Of the patients, 248 (49.6%) had I/II tumors, and 252 (50.4%) had III/IV tumors.

Oral mucosal cells were collected from each individual, and genomic DNA was extracted using the Chelex-100 method. A single nucleotide polymorphism (rs3735664 G>A) was amplified by polymerase chain reaction (PCR) and sequenced using an ABI 3730XL sequencer. The PCR conditions were pre-denaturation at 95 °C for 5 min; denaturation at 94 °C for 30 s; annealing at 58 °C for 30 s; extension at 72 °C for 30 s, 35 cycles; extension at 72 °C for 10 min. The PCR primer sequences were as follows: 5'-CGTCTCGGAGTGAATGACAG-3', 5'-AGGTACCACCTGGTCTCCTG-3'. The sequencing results were analyzed using Chromas software.

Tumor and adjacent non-tumor tissues were collected from 26 CRC patients who had not received preoperative radio- and chemotherapies. Human embryonic kidney cell line (HEK-293T) and CRC cell lines (HCT116 and HT29) were purchased from Shanghai EK-Bioscience Biotechnology Co., Ltd. *ELFN1-AS1* full-length sequence with the rs3735664

doi: 10.3967/bes2023.045

*This work was supported by the Opening Project of Jiangsu Province Engineering Research Center of Tumor Targeted Nano Diagnostic and Therapeutic Materials [No. JETNM202201].

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G (WT) and A (MUT) allele was inserted into the pcDNA3.1 vector to create *ELFN1-AS1* overexpression plasmids. An empty pcDNA3.1 vector was used as a negative control (NC). As per protocols, plasmids were transfected into HCT116 and HT29 cells using Lipofectamine 3000 (Invitrogen, USA). All cells were collected 48 hours later for further research. Total RNA was extracted from CRC tissues and cell lines using TRIzol reagent (Invitrogen, USA) according to the product manual, and cDNA was synthesized using a reverse transcription kit (Takara, Japan) according to the supplier's instructions. Thereafter, qRT-PCR was performed using an ABI Real-Time PCR system (Applied Biosystems, USA) and SYBR Green qPCR Master Mix (Takara, Japan). U6 and GAPDH were used as internal controls for miRNA and lncRNA, respectively. The relative expression levels of all genes were calculated using the $2^{-\Delta\Delta Ct}$ method. The sequences of the related primers are presented in Supplementary Table S2 available in www.besjournal.com. All participants provided written informed consent, and the study protocol was approved by the Ethical Committee of Yancheng First People's Hospital (2021-K- 117).

The case-control study found a significant association between *ELFN1-AS1* rs3735664 polymorphism and CRC susceptibility (Table 1). Individuals with rs3735664 AA genotype or carrying the A allele had a significantly lower risk of developing CRC compared to those with the GG genotype or carrying the G allele (*OR* = 0.53, 95% *CI* = 0.34−0.84, *P* = 0.006; *OR* = 0.75, 95% *CI* = 0.62−0.91, *P* = 0.003, respectively).

Furthermore, the study found a correlation between *ELFN1-AS1* rs3735664 polymorphism and the TNM stage in CRC patients (Table 2). Patients with the rs3735664 GA or AA genotype were less likely to develop stage III + IV tumors compared to those with the GG genotype (GA *vs.* GG: *OR* = 0.61, 95% *CI* = 0.42−0.89, *P* = 0.009; AA *vs.* GG: *OR* = 0.25, 95% *CI* = 0.11−0.56, *P* = 0.001). CRC patients carrying the rs3735664 A allele were less likely to develop stage III + IV tumors compared to CRC patients carrying the G allele (A *vs.* G: *OR* = 0.58, 95% *CI* = 0.44−0.77, *P* < 0.001).

The study also found a significant negative correlation between *ELFN1-AS1* expression and miR-1231 expression in colorectal tissues of rs3735664 GG and GA genotype (tumor tissues with GG genotype: *r* = −0.801, *P* = 0.001; adjacent non-tumor tissues with GG genotype: *r* = −0.844, *P* = 0.005; tumor tissues with GA genotype: *r* = −0.783, *P* = 0.012) (Table 3). The overexpression study showed that *ELFN1-AS1* with the rs3735664 G allele (WT) could reduce miR-1231 expression in CRC cells, while

Table 1. Association of *ELFN1-AS1* rs3735664 polymorphism with CRC susceptibility, *n* (%)

Genotype/allele	CRC patients (*n* = 500)	Cancer-free individuals (*n* = 500)	*OR* (95% *CI*)[a]	*P*[a]	Power
GG	268 (53.6)	230 (46.0)	1		
GA	197 (39.4)	213 (42.6)	0.79 (0.61–1.03)	0.083	0.514
AA	35 (7.0)	57 (11.4)	0.53 (0.34–0.84)	0.006	0.506
P_{HWE}[b]				0.472	
G	733 (73.3)	673 (67.3)	1		
A	267 (26.7)	327 (32.7)	0.75 (0.62–0.91)	0.003	0.488

Note. [a]: Adjusted for age and gender; [b]: P_{HWE} for the controls.

Table 2. Association of *ELFN1-AS1* rs3735664 polymorphism with the TNM stage in colorectal cancer, *n* (%)

Genotype/allele	III + IV (*n* = 252)	I + II (*n* = 248)	*OR* (95% *CI*)[a]	*P*[a]	Power
GG	153 (60.7)	115 (46.4)	1		
GA	90 (35.7)	107 (43.1)	0.61 (0.42–0.89)	0.009	0.508
AA	9 (3.6)	26 (10.5)	0.25 (0.11–0.56)	0.001	0.591
G	396 (78.6)	337 (67.9)	1		
A	108 (21.4)	159 (32.1)	0.58 (0.44–0.77)	< 0.001	0.695

Note. [a]: Adjusted for age and gender.

ELFN1-AS1 with A allele (MUT) was unable to do so (Supplementary Figure S2 available in www.besjournal.com). *ELFN1-AS1* sequence containing rs3735664 G (WT) and A (MUT) alleles was subcloned into the psiCHECK-2 vector. The recombinant dual-luciferase vectors (*ELFN1-AS1*-WT or *ELFN1-AS1*-MUT) were cotransfected into HEK-293T cells with miR-1231 mimic and miRNA NC, respectively. After 48 hours of transfection, the luciferase activity was measured using the Dual-Luciferase® Reporter Assay System (Promega, USA). The results of the luciferase assay showed that the rs3735664 A allele (MUT) was able to block the binding of *ELFN1-AS1* to miR-1231 (Supplementary Figure S3 available in www.besjournal.com). This suggests that *ELFN1-AS1* carrying the G allele may downregulate miR-1231 expression in CRC cells by adsorbing miR-1231. MiR-1231 has been linked to a negative association with tumor size, TNM stage, lymph node invasion, and poor prognosis in CRC patients[10]. The circTDRD3 promoted HIF1α expression by sponging miR-1231, which in turn contributes to the growth and metastasis of CRC[10]. Thus, the hypothesis is that the rs3735664 A allele may reduce the CRC-promoting role of *ELFN1-AS1* by blocking its adsorption to miR-1231, leading to a decrease in CRC risk and progression.

The current study provides insights into the role of lncRNA polymorphisms in CRC and reveals a new genetic marker for the disease. However, there are some limitations to be noted. Further research is needed to examine the interaction between the rs3735664 polymorphism and environmental risk factors and its effect on CRC susceptibility, and the relationship between the rs3735664 polymorphism and the survival prognosis of CRC patients is yet to be explored.

Despite these limitations, the findings suggest that the *ELFN1-AS1* rs3735664 polymorphism is associated with both CRC susceptibility and tumor stage in Chinese Han populations. The polymorphism holds promise as a biomarker for predicting CRC risk and progression.

Table 3. Correlation between *ELFN1-AS1* and miRNA expression in colorectal tissues with different rs3735664 genotypes

Tissue types	miRNAs	Genotype	Number of tissue	Correlation test	
				P	*r*
Adjacent non-tumor tissues	miR-599	GG	13	0.353	−0.192
		GA	9	0.512	−0.276
		AA	4	0.535	−0.463
	miR-1231	GG	13	0.005	−0.844
		GA	9	0.217	−0.451
		AA	4	0.193	−0.813
Tumor tissues	miR-599	GG	13	0.474	−0.374
		GA	9	0.612	−0.197
		AA	4	0.416	−0.592
	miR-1231	GG	13	0.001	−0.801
		GA	9	0.012	−0.783
		AA	4	0.135	−0.764

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Received: December 10, 2022;

Accepted: February 13, 2023

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