

High-expression of LncRNA MAFG-AS1 is associated with the prognostic of patients with colorectal cancer

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SUMMARY

OBJECTIVE: Long noncoding RNAs (lncRNAs) have been proven to exhibit distinct functions on the convoluted processes of tumor developments. Some studies on the biological functions of lncRNA MAFG-AS1 (MAFG-AS1) in cancers revealed that they may serve as an oncogene in some kinds of tumors, including colorectal cancer (CRC). However, little is known about the role of MAFG-AS1 in the prognostic of CRC.

METHODS: A public dataset was mined for the screening of dysregulated lncRNAs in CRC. Quantitative real-time reverse transcription-polymerase chain reaction (qPCR) was used to compare the levels of MAFG-AS1 between paired MAFG-AS1 specimens and normal adjacent tissues. The correlations between MAFG-AS1 and clinic pathological features in CRC were analyzed using the chi-square test. The log-rank test and Kaplan-Meier test were carried out to compare the survival time of patients with high and low expressions of MAFG-AS1. Cox regression was applied for univariate and multivariate assays to validate whether MAFG-AS1 could be an independent factor in the prognosis of CRC.

RESULTS: We found that the distinct upregulation of MAFG-AS1 in various tumors was a common event. MAFG-AS1 was distinctly up-regulated in CRC specimens compared to matched non-tumor specimens ($p < 0.01$). High MAFG-AS1 expressions were closely associated with depth of invasion ($p = 0.011$) and TNM stage ($p = 0.022$). Survival assays revealed that patients with high expression of MAFG-AS1 have a shorter overall survival ($p = 0.0030$) and disease-free survival ($p = 0.0002$). **CONCLUSIONS:** MAFG-AS1 can serve as a novel potential biomarker to predict CRC patients' survival time.

KEYWORDS: RNA, long noncoding. Colorectal neoplasms. Survival analysis.

INTRODUCTION

Colorectal cancer (CRC) is one of the most frequent cancers in human beings, accounting for 762,400 deaths annually worldwide¹⁻³. In recent years, the incidence and mortality rates of CRC have been evaluated to increase gradually in many countries,

especially in several low-income countries⁴. Despite the development of chemotherapeutic and surgical therapies, coupled with the advancements of novel molecular targeted therapeutics, have improved the five-year survival rate of CRC patients, the long-term

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survival of patients with advanced CRC remains poor^{5,6}. The probabilities of recurrence and metastasis are distinctly increased once the neoplasms enter the advanced stages, which is the main cause for the death of patients⁷. Thus, further knowledge of the potential mechanisms of CRC developments and an assessment of novel biological markers are required for the early detection and treatment of CRC.

Long non-coding RNAs (lncRNAs) are a set of RNAs with > 200 nucleotides in size and have limited protein-coding abilities due to the lack of an open read frame⁸. Recently, the imperative effects of lncRNAs in the modulation of gene expression have been frequently reported in a large number of studies, involved in the epigenetic regulation^{9,10}. Without a doubt, various biological progressions, such as chromosome replication, cytodifferentiation, life-cell metastasis and apoptosis, were influenced by the dysregulation of lncRNAs^{11,12}. With the development of sequencing technology and bioinformatics, growing abnormally-expressed protein^{s13} and lncRNAs have been periodically observed in various tumors involved in carcinogenesis and tumor progression, suggesting that several lncRNAs could function as novel biomarkers for early diagnosis, prognosis prediction, and sufficient therapeutic targets of malignancies^{14,15}. However, the investigation of further CRC-associated lncRNAs is warranted.

MAFG-AS1, a novel tumor-related lncRNA, has been reported to be abnormally expressed in several tumors, including CRC, breast carcinoma, and lung adenocarcinoma¹⁶⁻¹⁸. However, the potential function of MAFG-AS1 in tumors remains largely unclear. Recently, Cui et al.¹⁶ firstly reported MAFG-AS1 was an oncogenic lncRNA in CRC due to its frequent upregulation in CRC tissues and tumor-promotive roles in the proliferation and metastasis of CRC cells. However, the relative evidence supporting the over-expression of MAFG-AS1 was limited. In addition, its clinical significance in CRC patients remains to be further investigated.

METHODS

Patients and Tissue Samples

A total of 172 pairs of fresh CRC and adjacent non-tumor specimens were obtained from 172 patients with CRC, who underwent clinical surgery at our Hospital, Dongying People's Hospital, between April 2011 and July 2014. This cohort consisted of 72 females

and 100 males aged from 31 to 71 years (mean age was 43.7). All of the cases were histopathologically diagnosed as rectum carcinomas by two independent pathologists from our hospital. None of these patients had received radiotherapy or chemotherapy before surgery. To track the differential survivals, a five-year follow-up was conducted by straightforward telephone interviews or external checks until death or metastasis. The study protocol was approved by the ethics committee of the Dongying People's Hospital, and informed consent was obtained from all the patients.

RNA extraction and quantitative real-time reverse transcription-polymerase chain reaction (qPCR)

Total RNA from CRC specimens and matched normal samples were extracted using TRIzol reagent (Ming Technology, Hangzhou, Zhejiang, China) according to the company's handbook. The purity quotient of RNAs was examined using agarose gel electrophoresis, followed by DNAase for the elimination of impurities. A RevertAid™ First-Strand cDNA Synthesis Kit was purchased from Bichennan Biotechnology (Haidian, Beijing, China) and used to synthesize first-strand complementary DNA (cDNA) using a random primer. Then, an ABI Prism 7500 (Biosystems, Pudong, Shanghai, China) was used for the qRT-PCR assays, which were performed using a TaqMan MicroRNA Assay Kit (Biosystems, Pudong, Shanghai, China). The expression levels of MAFG-AS1 were analyzed by applying the comparative cycle threshold (CT) ($2^{-\Delta\Delta CT}$) methods with GAPDH as the endogenous control. The sequences of the PCR primers used in this study were as follows: MAFG-AS1 5'-ATGACGACCCCAATA-AAGGG-3' (sense); 5'-CACCGACATGGTTACCAGC-3' (antisense). GAPDH: 5'-GAAGGTGAAGGTCGGAGT-3' (sense); 5'-GAAGATGGTGACTGGGATTT-3' (antisense).

Statistical analysis

All data were analyzed using the SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). The associations between the MAFG-AS1 levels and clinicopathological factors were analyzed applying the chi-square test. The Student's *t*-test was applied to determine the statistical significance of the difference between CRC tissues and matched non-tumor specimens. The disease-free survival (DFS) and overall survival (OS) curves were plotted using the Kaplan-Meier methods, followed by the log-rank test to evaluate the statistical significances. Univariate and multivariate assays were

further used to explore the independent prognostic factors for CRC. Results were considered statistically significant at $p < 0.05$.

RESULTS

Increased expression of MAFG-AS1 in human CRC

The clinicopathological parameters of all 172 cases are presented in Table 1. To study the expression pattern of MAFG-AS1 in tumors, we searched an online system, the Gene Expression Profiling Interactive Analysis (GEPIA), which can be used to analyze the microarray data of gene expressions from TCGA datasets. As shown in Figure 1A, the distinct upregulation of MAFG-AS1 was observed in several different tumors, including CRC. Specifically, the expressions of MAFG-AS1 in 275 CRC specimens were distinctly higher than those in 349 non-tumor specimens ($p < 0.01$). In order to validate the above findings, we performed a RT-PCR to examine the levels of MAFG-AS1 in our cohort, further confirming the distinct overexpression of MAFG-AS1 in CRC tissues ($p < 0.01$).

TABLE 1. CORRELATION BETWEEN CLINICOPATHOLOGIC CHARACTERISTICS AND THE EXPRESSION OF MAFG-AS1 IN CRC.

Clinicopathological features	No. of cases	MAFG-AS1 expression		p-value
		High	Low	
Age				
<55	88	40	48	0.287
≥55	84	45	39	
Gender				0.268
Male	100	53	47	
Female	72	32	40	
Tumor location				0.886
Colon	91	47	44	
Rectum	81	38	34	
Tumor size				0.093
<5cm	102	45	57	
≥5cm	70	40	30	
Histology/differentiation				0.065
Well + Moderate	105	46	59	
Poor	67	39	28	
Depth of invasion				0.011
T1 + T2	117	50	67	
T3 + T4	55	35	20	
TNM stage				0.022
I-II	123	54	69	
III	49	31	18	

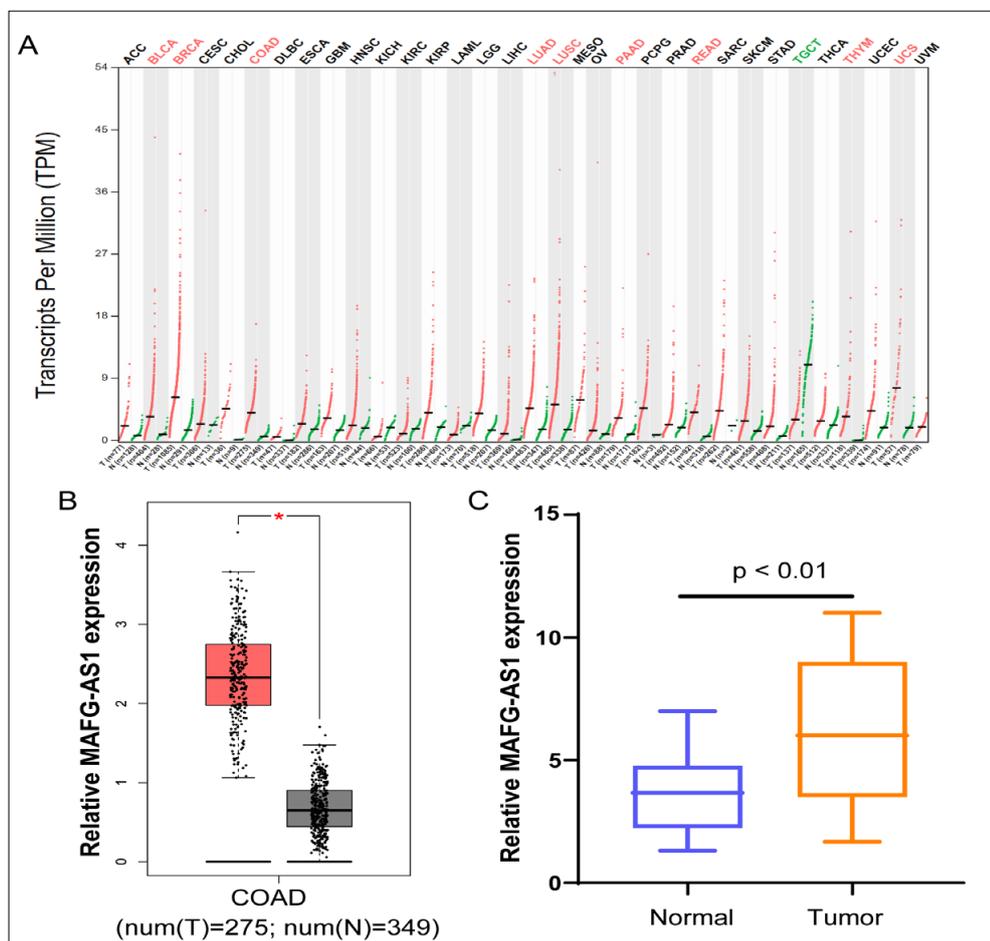


FIGURE 1. MAFG-AS1 EXPRESSION WAS UP-REGULATED IN CRC TISSUES

Clinical and pathological significance of MAFG-AS1 expression in CRC

To study the significance of MAFG-AS1 levels in the clinical progress of CRC patients, our group divided 172 patients into two groups: A high MAFG-AS1 expression group, $n = 85$, and a low MAFG-AS1 expression group, $n = 87$. Then, the chi-square test was applied to analyze the clinical data. Interestingly, in our cohort, high levels of MAFG-AS1 were positively associated with depth of invasion ($p = 0.011$) and TNM stage ($p = 0.022$). However, no distinct associations between MAFG-AS1 expressions and other clinical and pathological parameters were observed ($p > 0.05$).

Positive associations between MAFG-AS1 expressions and prognostic values of CRC patients

Given that the dysregulation of MAFG-AS1 was positively correlated with tumor invasion and advanced stages, we speculated that the levels of MAFG-AS1 may influence the long-term survival of CRC patients. The results of the Kaplan-Meier assays revealed that patients with high expressions of MAFG-AS1 had a shorter OS ($p = 0.0030$; Figure 2A) and DFS ($p = 0.002$; Figure 2B) as compared with the low-MAFG-AS1 group. Further univariate analysis indicated that depth of invasion, TNM stage, and MAFG-AS1 expression were distinctly associated with the OS and DFS of CRC patients (all $p > 0.05$). More importantly, multivariate assays for the above three factors demonstrated that MAFG-AS1 expression, in addition to depth of invasion and TNM

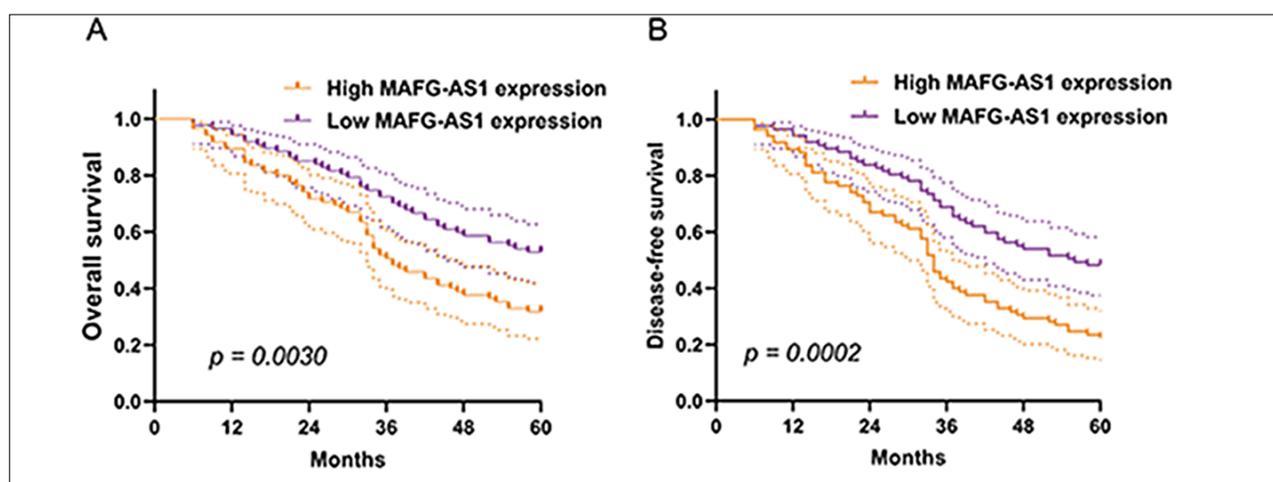
stage, were independent prognostic factors for both the OS (HR=2.737, 95% CI: 1.185-4.386, $p = 0.021$) and DFS (HR=2.869, 95% CI: 1.185-4.562, $p = 0.013$) of CRC patients.

DISCUSSION

In recent years, basic studies have highlighted the critical effects of lncRNAs in the progression of diseases, including tumors^{19,20}. In addition, more and more chip databases, such as the TCGA, GEO and COSMIC, displayed a large number of dysregulated lncRNAs in various types of tumors^{21,22}. On the other hand, the clinical values of lncRNAs acting as independent prognostic biomarkers have also been frequently reported. In this study, our attention was focused on a novel CRC-related lncRNA.

It is well known that lncRNAs act as imperative regulators in tumorigenesis by regulating numerous cellular biological phenotypes, such as cellular growth, invasive abilities, chemo-radioresistance, and so on²³. Recently, the dysregulation of MAFG-AS1 expression and its functional effects in several tumors have been reported in a few studies. For instance, the distinct overexpression of MAFG-AS1 was observed in breast cancer, and its knockdown was found to suppress the migration and invasion of breast cancer cells via the modulation of miRNA-339-5p/MMP15¹⁷. In lung cancer, a similar expression trend of MAFG-AS1 was also found in lung cancer. Functionally, the overexpression of MAFG-AS1 may result in increased abilities of migration and invasion in lung cancer cells by regulating the miRNA-339-5p/

FIGURE 2. SURVIVAL ANALYSIS OF 172 MAFG-AS1 PATIENTS BY KAPLAN-MEIER.



MMP15 axis¹⁸. A recent study by Ouyang et al.²⁴ indicated that the levels of MAFG-AS1 were increased in hepatocellular carcinoma specimens and cell lines. In their *in vitro* assays, the knockdown of MAFG-AS1 was demonstrated to inhibit the proliferation and metastasis of hepatocellular carcinoma cells via sponging miRNA-6852. Importantly, in CRC, MAFG-AS1 expression was reported to be upregulated, and its oncogenic roles in CRC proliferation and metastasis by modulating miR-147b/NDUFA4 were also observed¹⁶. Although MAFG-AS1 was also found to predict advanced clinical stages in CRC patients, the prognostic values of MAFG-AS1 in CRC patients have not been investigated.

In this study, we further explored the expression pattern of MAFG-AS1 in various tumors using “GEPIA” and found that the overexpression of MAFG-AS1 in tumor specimens was a common event, which highlighted the potential regulatory function of MAFG-AS1 in tumor progression. In our cohort, significantly increased levels of MAFG-AS1 were also observed. A previous study by Cui et al.¹⁶ also provided evidence that MAFG-AS1 was highly expressed in both CRC specimens and cell lines, which was consistent with our findings. Then, we analyzed the clinical and pathological data to study the clinical significance of MAFG-AS1 expression in CRC patients, finding that a high expression of MAFG-AS1 was strongly associated with depth of invasion and TNM stage, which was also in line with

previous findings that forced MAFG-AS1 expression could promote the metastasis of CRC cells¹⁶. Moreover, we first performed Kaplan-Meier assays to analyze the survival data with five years of follow-up and found that the 5-year OS and DFS of the high MAFG-AS1 expression group were distinctly shorter than those of the low MAFG-AS1 expression group. Further multivariate assays using the Cox regression model confirmed that a high MAFG-AS1 expression was an independent prognostic factor for CRC. However, given the small number of patients in our clinical assays, further research using a great number of CRC patients is required to further validate our findings.

CONCLUSIONS

Our findings initially suggested that MAFG-AS1 could serve as a potential target for treatment intervention and as a novel biomarker for CRC.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Author's Contribution

All authors contributed to the data analysis, drafting and revising of the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

RESUMO

OBJETIVO: RNAs longos não codificantes (lncRNAs) comprovadamente apresentam funções distintas no complicado processo de desenvolvimento de tumores. Alguns estudos sobre as funções biológicas dos lncRNA (MAFG-AS1) em cânceres revelaram que eles podem funcionar como um oncogene em alguns tipos de tumores, inclusive no câncer colorretal (CCR). No entanto, pouco é conhecido sobre o papel do MAFG-AS1 no prognóstico do CCR.

MÉTODOS: Um conjunto de dados públicos foi analisado em busca de lncRNAs desregulados em casos de CCR. Uma análise quantitativa de reação em cadeia de polimerase precedida de transcrição reversa (qPCR) em tempo real foi utilizada para comparar os níveis de MAFG-AS1 entre pares de amostras de MAFG-AS1 normal e tecidos adjacentes. As correlações entre MAFG-AS1 e características clínico-patológicas do CCR foram analisadas através do teste qui-quadrado. Os testes de Log-rank e Kaplan-Meier foram utilizados para comparar o tempo de sobrevivência de pacientes com expressões altas e baixas do MAFG-AS1. A regressão de Cox foi aplicada para ensaios uni e multivariados para validar se MAFG-AS1 poderia ser um fator independente no prognóstico do CCR.

RESULTADOS: Observamos que a regulação aumentada de MAFG-AS1 em vários tumores foi um evento comum. MAFG1 estava claramente com a regulação aumentada em espécimes de CCR quando comparado ao de espécimes equivalentes não-tumorais ($p < 0,01$). Altas expressões de MAFG-AS1 estavam associadas à profundidade de invasão ($p = 0,011$) e classificação no sistema TNM ($p = 0,022$). As análises de sobrevivência revelaram que pacientes com expressões elevadas do MAFG-AS1 tiveram uma diminuição significativa da sobrevivência global ($p = 0,0030$) e da sobrevivência livre de doença ($p = 0,0002$). **CONCLUSÃO:** MAFG-AS1 pode ser um novo potencial biomarcador para prever o tempo de sobrevivência de pacientes de CCR.

PALAVRAS-CHAVE: RNA longo não codificante. Neoplasias colorretais. Análise de sobrevivência.

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