A Long Noncoding RNA, lncRNA-ATB, Is Involved in the Progression and Prognosis of Colorectal Cancer

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Abstract. *Background/Aim: A long noncoding RNA (lncRNA) activated by transforming growth factor (TGF)-β (lncRNA-ATB) was recently described to promote the invasion-metastasis cascade in hepatocellular carcinoma. The aim of the present study was to clarify the clinicopathological role and prognostic relevance of lncRNA-ATB in colorectal cancer (CRC). Materials and Methods: lncRNA-ATB expression was evaluated by real-time reverse transcription polymerase chain reaction in 124 patients with CRC. Patients were divided into two groups based on the median lncRNA-ATB expression. Results: High lncRNA-ATB expression was significantly associated with greater tumor size, depth of tumor invasion, lymphatic invasion, vascular invasion, and lymph node metastasis. Patients of the high-lncRNA-ATB expression group had significantly poorer outcomes than those of the low-expression group. Additionally, levels of lncRNA-ATB expression were significantly higher in patients with hematogenous metastases. Conclusion: lncRNA-ATB may be involved in the progression of CRC and be a novel indicator of poor prognosis in patients with CRC.*

Colorectal cancer (CRC) is the third most common neoplasm worldwide (1). Despite major advancements in diagnostic and therapeutic approaches (*i.e.* chemotherapy and molecular-targeted therapy) for CRC, the prognosis of patients with distant metastases is unfavorable (2). Therefore, there is an urgent need to establish novel therapeutic strategies for treating patients with distant metastases arising from primary CRC.

Long noncoding RNA (lncRNA) is a type of noncoding RNA that consists of sequences longer than 200 nucleotides and can regulate chromosome structure and gene expression (3-7). Recently, studies have shown that lncRNA plays an important role in the development, growth, and progression of human carcinomas, acting as drivers of oncogenic functions through diverse mechanisms (7-12). The involvement of lncRNA in the progression and prognosis of CRC also has been demonstrated (4, 13). We previously reported that one lncRNA, homeobox (HOX) transcript antisense intergenic RNA (HOTAIR), regulates chromatin remodeling in cooperation with the polycomb-repressive complex 2 (4). Additionally, we found that HOTAIR was associated with prognosis in CRC (4). Therefore, our data supported the notion that lncRNAs function as drivers of cancer progression.

The epithelial-mesenchymal transition (EMT)-mediated invasion-metastasis cascade is well-established as the crucial phenomenon in cancer of the digestive organs, including CRC (14). Transforming growth factor (TGF)-β has been shown to induce EMT, leading to promotion of tumor progression, *i.e.* enhancement of proliferation, migration, and invasion (15). Recently, Yuan *et al.* demonstrated that an lncRNA activated by TGF-β, lncRNA activated by TGF-β (lncRNA-ATB), induces EMT, promotes tumor cell invasion through up-regulation of zinc finger E-box binding homeobox 1 (ZEB1) and ZEB2, and mediates distant metastasis by stabilization of interleukin (IL)-11 in hepatocellular carcinoma (16).

Therefore, in this retrospective study, we aimed to elucidate the clinicopathological and prognostic relevance of lncRNA-ATB in CRC.

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Materials and Methods

Patients. Between 1992 and 2002, 124 patients who underwent curative resection for CRC at our Institute and our affiliated hospital were enrolled in this study. The mean follow-up after initial surgery was 4.2±3.2 years. Any postoperative recurrence was entered into the database immediately when a patient died due to CRC, or if a recurrence was strongly suspected following analysis by diagnostic imaging, such as computed tomography or magnetic resonance imaging. The site of recurrence was recorded as a hematogenous metastasis or non-hematogenous metastasis. All clinicopathological data, including patient age, gender, histological grade, tumor size, depth of tumor invasion, lymphatic invasion, vascular invasion, lymph node metastasis, and clinical stage, were obtained from the database. Informed consent was obtained from each patient included in the study. All resected tumor samples were immediately collected, frozen in liquid nitrogen, and stored at –80˚C until RNA extraction.

Real-time reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from frozen CRC samples using a modified acid-guanidinium-phenol-chloroform procedure, and reverse transcription from 8 μl RNA was performed with random hexamer primers and Moloney murine leukemia virus (M-MLV) reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA, USA). Real-time RT-PCR was performed in a LightCycler 480 instrument (Roche Applied Science, Basel, Switzerland) using a LightCycler 480 Probes Master kit (Roche Applied Science). lncRNA-ATB primer sequences were as follows: sense, 5'-CTTCACCAGCACCCAGAGA-3' and antisense, 5'-AAGACAG AAAAACAGTTCCGAGTC-3'. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal housekeeping gene to normalize RNA concentrations between samples. The sequences of the GAPDH primers were as follows: sense, 5'-TTGGTATCGTGG AAGGACTCA-3' and antisense, 5'-TGTCATCATATTTGGCA GGTT-3'. The amplification conditions were as follows: 10 min at 95˚C followed by 45 cycles of 10 s at 95˚C and 30 s at 60˚C. All concentrations were calculated by the concentration of cDNA using Human Universal Reference Total RNA (Clontech, Palo Alto, CA, USA).

Statistical analysis. χ^2 tests and Student's *t*-tests were used for comparisons of lncRNA-ATB expression with clinicopathological findings. Survival curves were calculated by the Kaplan-Meier method, and differences between the curves were analyzed by the log-rank test. A Cox proportional hazards model was used in the multivariate survival analysis. These results were analyzed using the StatView-J 5.0 software program (SAS Institute Inc., NC, USA). *p*-Values less than 0.05 were considered statistically significant.

Results

Comparison of clinicopathological features between patients in the high-lncRNA-ATB and low-lncRNA-ATB expression groups. Firstly, we compared the clinicopathological findings of patients with high and low lncRNA-ATB expression, which were devided based on the median lncRNA-ATB expression (Table I). No significant differences were noted with respect to age, sex, or histological grade between the two groups.

Table I. *Comparative analysis of clinicopathological findings between patients in the group with low expression of long noncoding RNA activated by transforming growth factor-β (TGFβ) (lncRNA-ATB) and those in the group with high expression.*

	LncRNA-ATB expression				
	Low (n=62)		High (n=62)		*p*-Value
	n	%	n	%	
Age (years)					
<65	19	30.6	23	37.1	0.570
>66	43	69.4	39	62.9	
Sex					
Male	33	53.2	38	61.3	0.468
Female	29	46.8	24	38.7	
Histological grade					
Well/moderately differentiated	60	96.8	59	95.2	0.999
Other	2	3.2	3	4.8	
Tumor size					
<30 mm	44	71.0	49	87.5	0.041
>30 mm	18	29.0	7	12.5	
Depth of tumor invasion					
Up to the muscularis 27 propria	43.5	13	21.0	0.012	
Beyond the 35 subserosal layer	56.5	49	79.0		
Lymphatic invasion					
Absent	46	74.2	31	50.8	0.009
Present	16	25.8	30	49.2	
Vascular invasion					
Absent	55	88.7	45	73.8	0.039
Present	7	11.3	16	26.2	
Lymph node metastasis					
Absent	45	73.8	34	54.8	0.038
Present	16	26.2	28	45.2	
UICC stage					
0, I, II	46	74.2	34	54.8	0.038
III	16	25.8	28	45.2	

UICC: Union for International Cancer Control.

However, patients in the high lncRNA-ATB expression group had significantly larger tumor sizes and deeper invasion into the colorectal wall than patients in the low expression group (p=0.041 and p=0.012, respectively). Lymphatic invasion, vascular invasion and lymph node metastasis were more frequently observed in patients in the high-lncRNA-ATB expression group than those in the low-expression group (p=0.009, p=0.038, and p=0.039, respectively).

Recurrence-free survival and site of recurrence. The recurrence-free survival rates in patients with low lncRNA-ATB expression were 96.1%, 89.6%, and 89.6% at 1, 3, and 5 years, respectively, while those in patients with high lncRNA-ATB expression were 87.3%, 71.6%, and 71.6%,

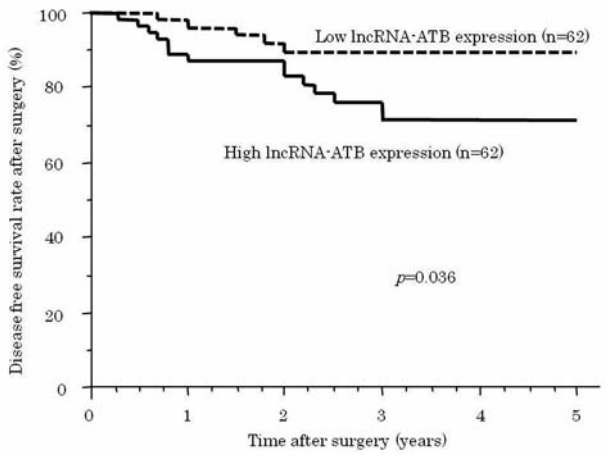

Figure 1. *Disease-free survival curve. Disease-free survival curve for patients with colorectal cancer with high long noncoding RNA activated by transforming growth factor-β (lncRNA-ATB) expression and low lncRNA-ATB expression.*

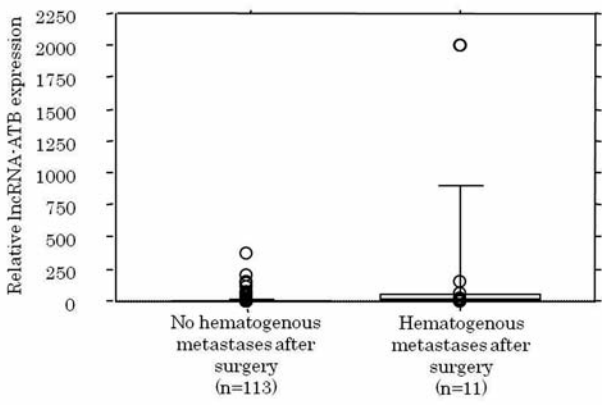

Figure 2. *Comparison of relative levels of long noncoding RNA activated by transforming growth factor-β (lncRNA-ATB) expression between patients with and those without hematogenous metastases after surgery (p=0.001).*

respectively. Patients in the high-lncRNA-ATB expression group had significantly poorer outcomes than those in the low-expression group in terms of recurrence-free survival (p=0.022) (Figure 1). Hematogenous metastases occurred in 14 out of 20 patients who experienced recurrence after initial surgery. Specifically, patients experienced recurrence in the liver (n=10), lung (n=3), spleen (n=1), adrenal gland (n=1), bone (n=1), and brain (n=1). Relative lncRNA-ATB expression levels were significantly higher in patients with hematogenous metastases after surgery than in those with non-hematogenous metastases or no recurrence after surgery (p=0.001) (Figure 2).

Discussion

Invasion and metastasis are the main causes of cancer-related mortality (17). Although great advancements have been made in diagnostic and chemotherapeutic approaches in recent years, the prognosis of patients with distant metastases arising from primary CRC remains unfavorable (2). Therefore, elucidation of the mechanisms controlling the invasion-metastasis cascade may provide insights into potential novel therapies for CRC. A recent study reported that lncRNA-ATB, a novel lncRNA induced by TGFβ, promotes the invasion of hepatoma cells through up-regulation of EMT-associated ZEB1 and ZEB2 by competitive binding with members of the microRNA (miR)-200 family and subsequent colonization of disseminated hepatoma cells at distant sites through signal tranducer and activator of transcription 3 (STAT3) signaling and IL11 production (16). EMT is involved in the invasion, metastasis, and prognosis of various types of cancers, including CRC (18, 19). Because this mechanism is well-established as the crucial event required for tumor metastasis in cancer of the digestive organs, including CRC (14), we further pursued elucidation of the functional role of lncRNA-ATB in CRC.

miR200c is epigenetically regulated at the invasive front of CRC and up-regulates EMT-related genes (*e.g.* ZEB1, ZEB2, and fms-like tyrosine kinase (FLT1) following distant metastasis (20). Calon *et al*. reported that secretion of IL11 induced by TGFβ was necessary for the acquisition of metastatic potential through crosstalk between cancer cells and the microenvironment in patients with CRC (21). In the current study, lncRNA-ATB expression was significantly associated with tumor size, depth of tumor invasion, lymphatic invasion, vascular invasion, and lymph node metastasis. In addition, levels of lncRNA-ATB expression were significantly higher in patients with recurrent hematogenous metastases. Therefore, consistent with these previous reports, our study supports the notion that lncRNA-ATB promotes the invasion and metastasis of CRC. However, a previous microarray analysis revealed that 16 lncRNAs were differentially expressed between metastatic CRC tissues and non-metastatic CRC tissues (22); lncRNA-ATB (gene symbol: AL589182.3) (16) was not one of these. Further studies are required to elucidate the mechanisms of the invasion-metastasis cascade, including those not associated with lncRNA-ATB.

In the present study, we demonstrated, as for as we are aware for the first time, that lncRNA-ATB may be a new prospective biomarker for invasion and metastasis of CRC. Additionally, high expression of lncRNA-ATB was found to be associated with recurrence after surgery, particularly hematogenous metastasis. Therefore, lncRNA-ATB may represent a new therapeutic target for controlling the invasion-

metastasis cascade of CRC. Further studies are required to determine how lncRNA-ATB is involved in the molecular mechanisms of the invasion-metastasis cascade in CRC.

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