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RESEARCH ARTICLE

Brain-derived neurotrophic factor downregulation in gastric cancer

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Abstract

The brain-derived neurotrophic factor (BDNF) is a certain type of growth factor that participates in the correct construction of the brain. Moreover, some reports have shown its participation in the tumorigenesis process. A long noncoding RNA known as *BDNF-antisense* (*BDNF-AS*) is shown to be transcribed from the antisense direction of the *BDNF* gene and control its expression. In the current study, we compared expression levels of *BDNF* and its antisense in gastric cancer tissues and adjacent noncancerous tissues (ANCTs) using quantitative real-time polymerase chain reaction. Expression of both genes tended to decrease in gastric cancer tissues in comparison with ANCTs (expression ratio = 0.4 and $P = .06$ for *BDNF*; expression ratio = 0.35 and $P = .05$ for *BDNF-AS*, respectively). Relative transcript levels of both genes were remarkably associated with the site of primary tumor in a way that all cardia tumors had low levels of both *BDNF* and *BDNF-AS* in comparison with their paired ANCTs ($P = .002$ and $P = <.001$). We also found higher amounts of both genes in malignant samples obtained from older patients ($P = .01$ and $P = .03$ for *BDNF* and *BDNF-AS*, respectively). Besides, *BDNF* expression was higher in tumors with lymphatic/vascular invasion ($P = .01$). There was also a trend toward upregulation of *BDNF-AS* in tumors with lymphatic/vascular invasion ($P = .05$). The current study underscores the role of *BDNF* and *BDNF-AS* in the pathogenic process leading to gastric cancer.

KEYWORDS

BDNF, BDNF-AS, gastric cancer

1 | INTRODUCTION

The brain-derived neurotrophic factor (BDNF) is a type of growth factor that participates in the correct formation of the brain. Apart from this crucial role, BDNF is involved in the tumorigenesis process. However, data regarding its role in this process are inconsistent. On one hand, BDNF expression has been elevated in several kinds of human malignancies in association with aggressive tumor behaviors and unresponsiveness to conventional chemotherapeutic regimens.[1] Mechanistically, the interface between BDNF and tyrosine kinase receptor B initiates a signaling flow recognized by high function of the phosphatidylinositol 3-kinase/protein kinase B pathway. Such interaction also results in overexpression of genes that increase cell migration and survival while inhibit the apoptosis.[2,3] Moreover, BDNF signaling route induces the epidermal growth factor receptor signaling in an independent manner from the endogenous epidermal growth factor ligand.[4] On the basis of these observations,

BDNF antagonism is expected to decrease cancer burden in clinical settings.[1] On the other hand, higher expression of BDNF in the hypothalamus has been associated with induction of immune cells that have activity against tumor cells and decreasing the function of numerous molecules that are anticipated to confer irresponsiveness of cancer cells to chemotherapy.[2] Consistent with the latter function, the serum levels of BDNF have been considerably decreased in colorectal cancer patients relative to healthy subjects.[5] Based on the intricacy of BDNF function in tumorigenesis, research on the function of BDNF is ongoing. To add to this complexity, a long noncoding RNA (lncRNA) has been identified that is located in the antisense direction from *BDNF* and probably participates in the modulation of *BDNF* transcription.[6] This lncRNA has been shown to be downregulated in some malignancies such as retinoblastoma[7] and prostate cancer.[8] However, the expression pattern and significance of this lncRNA in gastric cancer is largely unidentified. In the current research, we aimed at the identification of transcript levels of *BDNF* and its antisense transcript in gastric cancer tissues and their adjacent noncancerous tissues (ANCTs) in association with tumoral features to find their relevance with the carcinogenesis process in this organ.

2 | MATERIALS AND METHODS

2.1 | Patients

The present research was accomplished on tissue specimens obtained from 30 patients with histopathologically defined gastric cancer. Both tumoral and ANCTs were obtained during surgical resection of gastric tumor. Patients had not received any kinds of chemo/radiotherapy. All tissue samples were examined by pathologists to appraise the existence of tumoral cells. The study protocol was accepted by the Ethical Committee of Shahid Beheshti University of Medical Sciences. All patients signed written informed consent forms.

2.2 | Expression analysis

Total RNA was drawn out from all specimens using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. Complementary DNA (cDNA) was produced from 50 to 75 ng of RNA samples using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Expressions of *BDNF* and the antisense RNA were assessed and normalized to those of *HPRT1* in the Rotor Gene 6000 Machine by means of TaqMan Universal PCR Master Mix (Applied Biosystems). The detailed information of primers and probes is presented in Table 1.

2.3 | Evaluation of the *Helicobacter pylori* presence in specimens

The *Helicobacter pylori* presence in tissues was assessed by real-time polymerase chain reaction using primers against *H. pylori* 16s rRNA (F: AGCGTTACTCGGAA TCACTG; R: CACATACCTCTCACACACTC). The final concentration of each primer was 0.2 pmol/μL. Reactions were performed on 100 ng of synthesized cDNA. The polymerase chain reaction program comprised the succeeding phases: 95°C for 15 minutes and then 95°C for 20 seconds, and 60°C for 60 seconds for 40 cycles. Melt curve analysis was also performed.

2.4 | Statistical analysis

The REST 2009 software was applied for valuation of relative expression levels of genes in malignant tissues vs ANCTs. The change in transcript quantities between paired tumoral tissues and ANCTs was assessed using the Student paired *t* test. The association between tumor characteristics and transcription of genes was measured using the χ^2 or Mann-Whitney tests. The correlation between the transcription level of *BDNF* and *BDNF-AS* was evaluated using the regression model. *P* values smaller than .05 were considered significant.

TABLE 1 Sequences of primers and probes used in the current study

Gene name	Primer and probe sequence	Product length
HPRT1	F: AGCCTAAGATGAGAGTTC R: CACAGAACTAGAACATTGATA FAM -CATCTGGAGTCCTATTGACATCGC- TAMRA	88
BDNF	F: GATGCTGCAAACATGTCCATGAG R: TTTTGTCTGCCGCCGTTACC FAM-CCACTCTGACCCTGCCCGCCGA-TAMRA	109
BDNF-AS	GTGGGTCCATTCCGTGTGTG AGCTGGTGCAGGTATCAGATTAG FAM-TCCAGTGGAAACGCTGCCTCACCA-TAMRA	97

3 | RESULTS

3.1 | Overall information of study participant

General characteristics of study participants are presented in Table 2.

3.2 | Relative expression of genes in malignant samples compared with ANCTs

Expression levels of both genes tended to decrease in gastric cancer specimens in comparison with ANCTs (expression ratio = 0.4 and $P = .06$ for *BDNF*; expression ratio = 0.35 and $P = .05$ for *BDNF-AS*, respectively). Figure 1 demonstrates the $-\Delta C_T$ values (CT *HPRT1*- CT target gene) in malignant specimens and ANCTs.

TABLE 2 General demographic and clinical data of study participants

Variables		Values
Age, mean ± SD (range)		42.53 ± 10.1 (14-55)
Sex	Male	78.6%
	Female	21.4%
Site of primary tumor	Cardia	41.4%
	Antrum	31%
	Body	27.6%
Histologic grade	2	37.5%
	3	58.3%
	4	4.2%
Lymphatic invasion	Yes	82.8%
	No	17.2%
Vascular invasion	Yes	82.8%
	No	17.2%
Peritoneal invasion	Yes	62.1%
	No	37.9%
TNM stage	I	3.4%
	II	31%
	III	44.8%
	IV	20.8%
Histological form	Intestinal	46.7%
	Diffuse	53.3%
H. pylori infection	Positive	50%
	Negative	50%
Smoking	Never smoker	50%
	Current smoker	13.6%
	Ex-smoker	36.4%

3.3 | Association between tumor features and relative expression of genes

Relative transcript levels of both genes were remarkably associated with the site of the primary tumor in a way that all cardia tumors had low levels of both genes in comparison with their paired ANCTs ($P = .002$ and $P \leq .001$). Other features were not associated with the expression of either gene. Table 3 demonstrates the association between transcript levels of genes in gastric tumor specimens in comparison with ANCTs and clinical data.

Furthermore, we compared transcript levels of genes between discrete groups of patients and found higher levels of both genes in older patients ($P = .01$ and $P = .03$ for *BDNF* and *BDNF-AS*, respectively). Besides, *BDNF* expression was higher in tumors with lymphatic/vascular invasion ($P = .01$). There was also a trend toward upregulation of *BDNF-AS* in tumors with lymphatic/vascular invasion ($P = .05$). Table 4 displays associations between expression of genes in malignant samples and patients' characteristics.

3.4 | Correlation between transcript amounts of *BDNF* and its antisense RNA

Remarkable pairwise correlations were recognized between transcript levels of *BDNF* and its antisense RNA in both malignant specimens and ANCTs (Figure 2A,B, respectively).

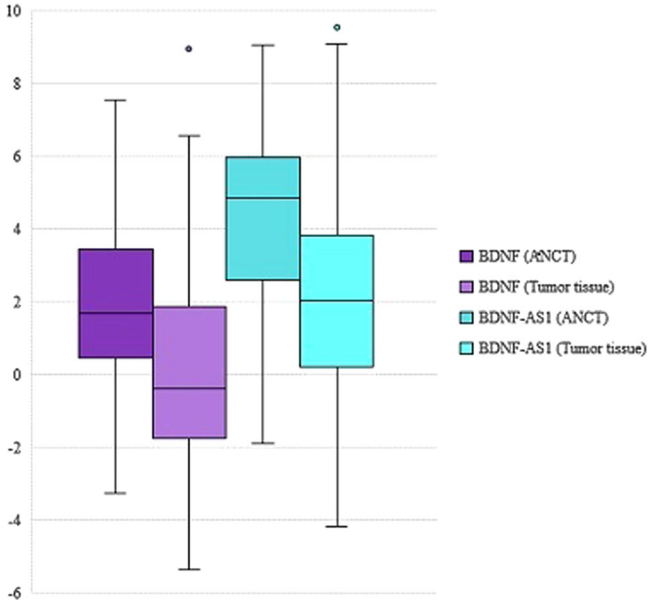

FIGURE 1 shows the $-\Delta C_t$ values (CT HPRT1- CT target gene) in tumoral tissues and ANCTs. ANCTs, adjacent noncancerous tissues; BDNF, brain-derived neurotrophic factor

TABLE 3 The results of association analysis between relative expressions of *BDNF* and *BDNF-AS* in gastric tumor specimens compared with ANCTs and clinical data (up/down regulation of genes was described based on the relative expression of each gene in tumoral tissue compared with the corresponding ANCT)

	BDNF **upregulation**	***BDNF*** **downregulation**	***P* value**	***BDNF-AS1*** **upregulation**	***BDNF-AS1*** **downregulation**	***P* value**
Age			1			.67
>50	7 (33.3%)	14 (66.7%)		7 (33.3%)	14 (66.7%)	
≤50	2 (28.6%)	5 (71.4%)		3 (42.9%)	4 (57.1%)	
Sex			.14			.63
Female	0 (0%)	6 (100%)		1 (16. 7%)	5 (83.3%)	
Male	8 (36.4%)	14 (63.6%)		8 (36.4%)	14 (63.6%)	
Site of primary			.002			<.001
Cardia	0 (0%)	12 (100%)		0 (0%)	12 (100%)	
Antrum	6 (66.7%)	3 (33.3%)		8 (88.9%)	1 (11.1%)	
Body	3 (37.5%)	5 (62.5%)		2 (25%)	6 (75%)	
Histology grade			1			.29
2	3 (33.3%)	6 (66.7%)		2 (22.2%)	7 (77.8%)	
3	5 (35.7%)	9 (64.3%)		7 (50%)	7 (50%)	
4	0 (0%)	1 (100%)		0 (0%)	1 (100%)	
Lymphatic invasion			.63			1
Yes	7 (29.2%)	17 (70.8%)		8 (33.3%)	16 (66.7%)	
No	2 (40%)	3 (60%)		2 (40%)	3 (60%)	
Vascular invasion			.63			1
Yes	7 (29.2%)	17 (70.8%)		8 (33.3%)	16 (66.7%)	
No	2 (40%)	3 (60%)		2 (40%)	3 (60%)	
Peritoneal invasion			1			1
Yes	6 (33.3%)	12 (66.7%)		6 (33.3%)	12 (66.7%)	
No	3 (27.3%)	8 (72.7%)		4 (36.4%)	7 (63.6%)	
Pathological T			.41			1
T2b	0 (0%)	4 (100%)		1 (25%)	3 (75%)	
T3	6 (35.3%)	11 (64.7%)		6 (35.3%)	11 (64.7%)	
T4	2 (33.3%)	4 (66.7%)		2 (33.3%)	4 (66.7%)	
Pathological N			.46			.2
N0	3 (33.3%)	6 (66.7%)		2 (22.2%)	7 (77.8%)	
N1	2 (22.2%)	7 (77.8%)		3 (33.3%)	6 (66.7%)	
N2	4 (50%)	4 (50%)		5 (62.5%)	3 (37.5%)	
N3	0 (0%)	3 (100%)		0 (0%)	3 (100%)	
TNM staging			1			.74
I	0 (0%)	1 (100%)		0 (0%)	1 (100%)	
II	3 (33.3%)	6 (66.7%)		2 (22.2%)	7 (77.8%)	
III	4 (30.8%)	9 (69.2%)		6 (46.2%)	7 (53.8%)	
IV	2 (33.3%)	4 (66.7%)		2 (33.3%)	4 (66.7%)	
Histological form			.69			.79
Intestinal	5 (35.7%)	9 (64.3%)		5 (35.7%)	9 (64.3%)	
Diffuse	4 (25%)	12 (75%)		5 (31.3%)	11 (68.7%)	
H. pylori infection			.42			.7
Positive	3 (20%)	12 (80%)		4 (26.7%)	11 (73.3%)	
Negative	6 (40%)	9 (60%)		6 (40%)	9 (60%)	
Smoking			.15			1
Nonsmoker	4 (36.4%)	7 (63.6%)		4 (36.4%)	7 (63.6%)	
Smoker	2 (66.7%)	1 (33.3%)		1 (33.3%)	2 (66.7%)	
Ex-smoker	1 (12.5%)	7 (87.5%)		2 (25%)	6 (75%)	

Abbreviations: ANCTs, adjacent noncancerous tissues; BDNF, brain-derived neurotrophic factor.

TABLE 4 The results of association analysis between transcript levels of genes in tumoral tissues and patients' characteristics (mean [standard deviation]) values of efficiency ^CT reference gene-efficiency ^CT target gene are presented)

	BDNF	*P* value	*BDNF-AS1*	*P* value
Age				
<50 y old vs ≥50 y old	16.26 (56.29) vs 82.98 (131.64)	.01	142.12 (636.3) vs 213.66 (517.14)	.03
Lymphatic invasion				
Yes vs no	38.41 (89.85) vs 0.15 (0.18)	.01	186.65 (646.39) vs 1. 17 (2.3)	.05
Vascular invasion				
Yes vs no	38.41 (89.85) vs 0.15 (0.18)	.01	186.65 (646.39) vs 1. 17 (2.3)	.05
H. pylori infection				
Positive vs negative	22.99 (66.77) vs 46.34 (97.21)	.71	197.77 (752.87) vs 101.26 (355.63)	.77
Tumor grade				
Grade 2 vs 3 and 4	32.23 (84.85) vs 41.87 (98.56)	1	326.52 (972.21) vs 102.68 (355.24)	.44

Abbreviation: BDNF, brain-derived neurotrophic factor.

Figure 2 Pairwise correlation between transcript amounts of *BDNF* and its antisense RNA in gastric cancer samples (A) and ANCTs (B).

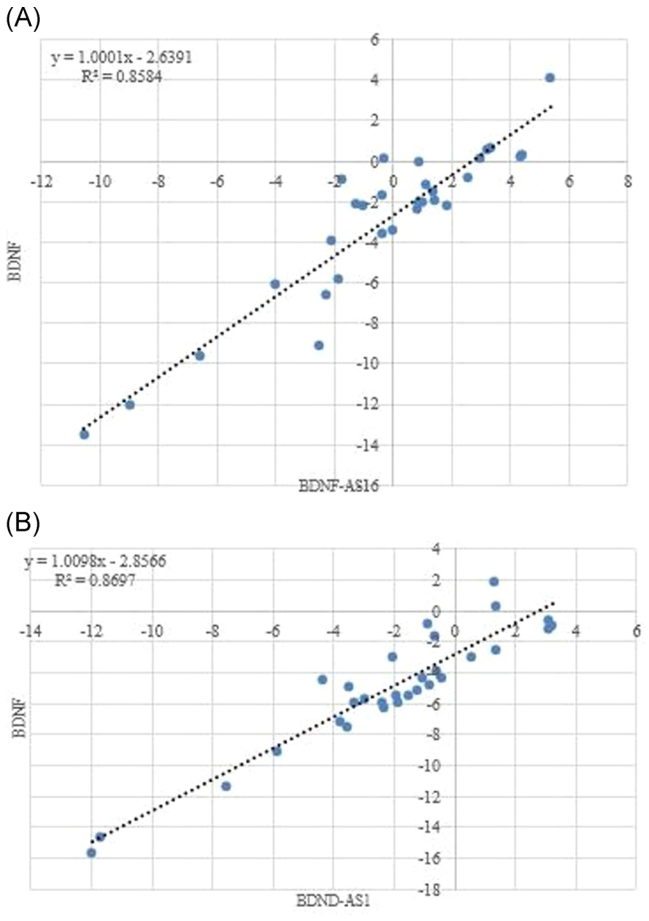

FIGURE 2 Pairwise correlation between expression levels of BDNF and BDNF-AS in gastric cancer tissues (A) and ANCTs (B). 99 × 156 mm (96 × 96 DPI). ANCTs, adjacent noncancerous tissues; BDNF, brain-derived neurotrophic factor

4 | DISCUSSION

In the current research, we quantified expression levels of *BDNF* and *BDNF-AS* in both tumoral samples and ANCTs obtained from patients with gastric cancer. The results showed a tendency toward downregulation of *BDNF* in tumoral tissues. A previous study reported higher BDNF expression at the invasive edge of initial tumors in comparison with that in the middle of the tumor and adjacent noncancerous mucosa.[9] The inconsistency between these studies might be due to dissimilarities in the characteristics of patients especially regarding the age of study participants which was significantly higher in the Okugawa et al[9] study compared with the current study. We also discovered greater amounts of *BDNF* expression in older individuals which contradict the Okugawa et al[9] study where BDNF overexpression was associated with younger age. Such contradiction might be explained by the site of gene expression assessment which was at the tumor invasive front and whole tumor bulk in the Okugawa et al[9] research and the current research, respectively. Yet, in line with the mentioned research,[9] we reported higher *BDNF* levels in tumors with lymphatic/vascular invasions. This pattern of expression is in discordant with the observed downregulation of *BDNF* in tumoral tissues compared with ANCTs. However, previous studies have also reported overexpression of some tumor suppressor genes in invasive gastric lesions. For instance, Maehara et al[10] have reported overexpression of p53 protein in gastric tumor tissues with vascular invasion and reported p53 overexpression as an indicative of metastasis to remote organs. In line with the observed pattern of *BDNF* expression in tumors with lymphatic/vascular invasion, Jiffar et al[11] have shown that BDNF controls the process of lymphovascular metastasis via a fibroblast-regulated

paracrine route in the microenvironment of cancer. So assessment of expression of this gene in certain components of microenvironment as well as tumoral cells is needed to elaborate the underlying mechanism of the observed pattern of its expression.

We also reported a trend toward downregulation of *BDNF-AS* in tumoral samples in comparison with nontumoral tissues which is in accordance with the expression pattern of this lncRNA in other malignancies.[7,8] The observed higher level of this lncRNA in older patients is consistent with the previously reported age-related dissimilarities in clinicopathological features. Notably, such pattern of expression is in accordance with the observed more aggressive behavior of gastric cancer in younger patients.[12,13]

Most importantly, relative transcript levels of both genes were remarkably associated with the site of primary tumor in a way that all cardia tumors had low levels of both *BDNF* and its antisense RNA in comparison with their paired nontumoral tissues. These results indicate that expression levels of these genes might be applied for differentiation of malignant status in samples obtained from the cardia region. An alternative explanation for this observation is that cardia is the region of the glands that principally secrete mucus but antrum has pyloric glands that secrete gastrin. Finally, the fundic glands secrete hydrochloric acid and intrinsic factor.[14] So, the observed difference in the expression pattern of these genes might be due to the difference in gastric mucosa structure or biologic environment. Expression analysis in larger samples of tumors from this region is needed to verify this speculation.

We recently compared expression levels of these genes in breast cancer samples and their nontumoral specimens and detected no remarkable difference in their expression between these two sets of samples. However, we reported significant associations between expression of the antisense RNA and both tumor grade and mitotic rate, and between *BDNF* level and estrogen receptor status.[15] Taken together, the associations between transcript amounts of mentioned genes and clinical characteristics in these two types of cancers indicate possible roles for these genes in human malignancies. However, these genes might affect distinct pathways or cascades in each malignancy type.

The positive correlation between transcript amounts of mentioned genes in the current study is consistent with our recent work in breast samples.[15] These observations in both gastric and breast tissues are in contrast with the previously suggested putative function of *BDNF-AS* in inhibition of *BDNF* transcription.[6] Such inconsistencies might be explained by distinct roles of lncRNAs in the individual pathophysiological conditions. However, this hypothesis should be verified in future researches.

In summary, in the present research, we demonstrated the downregulation of *BDNF* and a trend toward this pattern for *BDNF-AS* in gastric cancer specimens in comparison with ANCTs in association with some clinicopathological features. These findings add a layer of complexity in the previously reported roles of these genes in the tumorigenesis process and the regulatory role of *BDNF-AS* on *BDNF*. Based on the inconsistencies between the results of studies, it is necessary to assess the expression of BDNF in certain cancer types before the establishment of any targeted therapy against it.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTIONS

FE and MSK performed the laboratory test. MT and SGH wrote the manuscript and supervised the study. HB and VKO analyzed the data. MCG collected the data.

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