

# LncRNA DQ786243 expression as a biomarker for assessing prognosis in patients with gastric cancer

M.-H. ZHANG, Y. YANG, Y. ZHAO, H.-B. WEI, Y.-Q. MA, C.-J. YANG, X.-J. ZHANG, Y.-L. SUN

The Third Internal Medicine Oncology, Chifeng City Hospital, Chifeng Clinical Medicine College of Inner Mongolia Medical University, Chifeng, Inner Mongolia Autonomous Region, China

**Abstract. – OBJECTIVE:** Recent studies have revealed that long noncoding RNA DQ786243 (DQ786243) plays an important role in the development of gastric cancer (GC) and colorectal cancer. However, the expression and function of DQ786243 in GC patients remain largely unknown. The purpose of our study was to investigate the clinical significance of DQ786243 expression in GC.

**PATIENTS AND METHODS:** qRT-PCR was used to examine DQ786243 expression in 172 paired GC samples and matched adjacent normal tissues. Besides, the relationship between DQ786243 expression and clinicopathologic characteristics was analyzed. Overall survival (OS) and progression-free survival (PFS) curves of patients in subgroups were constructed by the Kaplan-Meier method. Multivariate regression analyses were performed to analyze independent factors affecting prognosis.

**RESULTS:** We found a significant up-regulation of DQ786243 in GC tissues compared to normal tissues ( $p < 0.01$ ). High DQ786243 expression was closely associated with invasion depth ( $p = 0.006$ ), TNM stage ( $p = 0.009$ ) and lymphatic metastasis ( $p = 0.017$ ). Moreover, Kaplan-Meier analysis showed that patients with DQ786243 high expression tumors had worse OS ( $p = 0.0012$ ) and PFS ( $p = 0.0002$ ) compared to patients with DQ786243 low expression tumors. Finally, multivariate analysis showed that DQ786243 was a significant and independent prognostic predictor for both OS ( $p = 0.002$ ) and PFS ( $p = 0.001$ ) of GC patients.

**CONCLUSIONS:** Our data suggest, for the first time, that the evaluation of the DQ786243 expression in GC tissues is a useful tool for predicting prognosis of GC.

*Key Words:*

Long noncoding RNA DQ786243, Gastric cancer, Prognosis.

## Introduction

Gastric cancer (GC) is one of the most frequently diagnosed cancers worldwide, causing more than 800,000 deaths all over the world each year<sup>1,2</sup>. Environmental and genetic factors play important role in gastric carcinogenesis<sup>3</sup>. Although significant advancement has been achieved in surgical techniques and adjuvant treatment, most of the GC patients have a poor prognosis. The overall 5-year relative survival rate is about 25% in China<sup>4,5</sup>. Generally speaking, patients with GC suffer from poor outcome due to the massive invasion and metastasis of tumor cells<sup>6</sup>. The clinical assay shows that the clinical prognosis of GC patients may be strongly high in early diagnosis. Therefore, there is an urgent requirement to identify putative diagnostic markers and prognostic factors for the treatment of GC patients.

Long noncoding RNA (lncRNA) is a class of RNA over 200 nucleotides in length with no protein-coding potential<sup>7</sup>. For a long time, lncRNAs are considered to be transcriptional noise. However, emerging evidence reveals that lncRNAs-mediated biological functions play significant roles in a variety of cellular processes, such as cell proliferation, differentiation, apoptosis, and cancer metastasis<sup>8</sup>. Mechanism studies show that lncRNAs exert its function by DNA damage, angiogenesis and microRNA silencing<sup>9-11</sup>. Notably, lncRNA was found to function as either oncogene or anti-oncogene. Given the important role of lncRNAs in GC progression, the value of lncRNAs functioning as potential biomarkers draws researcher's attention. Several lncRNAs such as lncRNA NEAT1, lncRNA TUSC7, and lncRNA AFAP1-AS1 were identified to be associated with prognosis of GC patients<sup>12-14</sup>.

DQ786243, a newly identified lncRNA, was firstly found to be highly expressed in hepatocellular carcinoma<sup>15</sup>. It was reported that DQ786243 played an important role in the progression of Crohn's disease<sup>16</sup>. Subsequently, up-regulation of DQ786243 was reported in GC and colorectal cancer patients<sup>17,18</sup>. These findings indicated DQ786243 as an important regulator in GC. However, the prognostic significance of DQ786243 in GC has not been discussed.

## Patients and Methods

### Tissue Samples and Patient Data

We selected tissue samples from 172 cases of GC, along with the corresponding noncancerous tissues, from patients diagnosed at the Chi Feng Clinic of Inner Mongolia Medical University and Chifeng Hospital between 2007 and 2012. There were 109 men and 63 women, with an average age of 57 years (range: 31-88 years). All the patients did not receive any pre-operative chemotherapy or radiotherapy before the surgery. HCC diagnosis was based on World Health Organization criteria. Tumor differentiation was defined ac-

cording to the Edmondson grading system. The main clinical and pathological parameters of the patients are shown in Table I. This study approval was obtained from the Ethics Committee of the Chi Feng Clinic of Inner Mongolia Medical University and Chifeng Hospital. All patients gave written informed consent to participate in the present study.

### RNA Extraction and Quantitative Real-Time PCR

Total RNA was isolated from cells and tissues using TRIzol (Invitrogen, Carlsbad, CA, USA). cDNA was generated from RNA using SuperScript III (Invitrogen, Carlsbad, CA, USA). RT-PCR was performed with the 7300 sequence detection system (Biosystems, Dongcheng, Beijing, China) using SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA). U6 was used as an endogenous control. The qRT-PCR specific primers were purchased from Ambion (Xuhui, Shanghai, China). The qRT-PCR reactions were performed in triplicate. Relative expression of DQ786243 was measured by standard curves and quantified using the  $2^{-\Delta\Delta Ct}$  method.

**Table I.** Correlation of clinicopathological features of GC with DQ786243 expression levels.

Characteristics	All cases	DQ786243 expression		p-value
		High	Low	
Age				0.559
≥ 60	78	40	38	
< 60	94	44	50	
Gender				0.233
Male	109	57	52	
Female	63	27	36	
Differentiation				0.093
Well-moderate	117	52	65	
Poor	55	32	23	
Lauren type				0.382
Intestinal	70	37	33	
Diffuse and mixed	102	47	55	
Tumor size				0.179
≥ 5 cm	59	33	26	
< 5 cm	113	51	62	
Invasion depth				0.006
T1, T2	106	43	63	
T3, T4	66	41	25	
TNM stage				0.009
I/II	111	46	65	
III/IV	61	38	23	
Lymphatic metastasis				0.017
Negative	123	53	70	
Positive	49	31	18	

**Statistical Analysis**

Statistical analysis was performed using SPSS software, version 19.0 (SPSS Inc., Chicago, IL, USA). Student's *t*-test is applied for comparison of the two groups. The correlation between DQ786243 expression and clinical parameters was achieved with Spearman's correlation analysis. Survival distributions were estimated by the Kaplan-Meier method, and differences in survival were evaluated using the log-rank test. A Cox regression model was used for multivariate analysis of prognostic parameters. *p*-values < 0.05 were considered statistically significant.

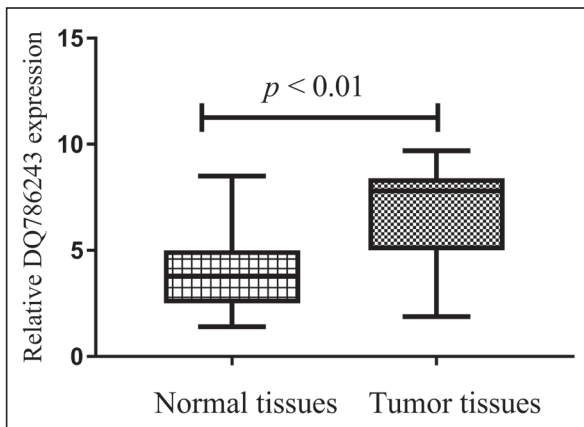
**Results**

**Expression of DQ786243 in the GC Tissues and Adjacent Normal Tissues**

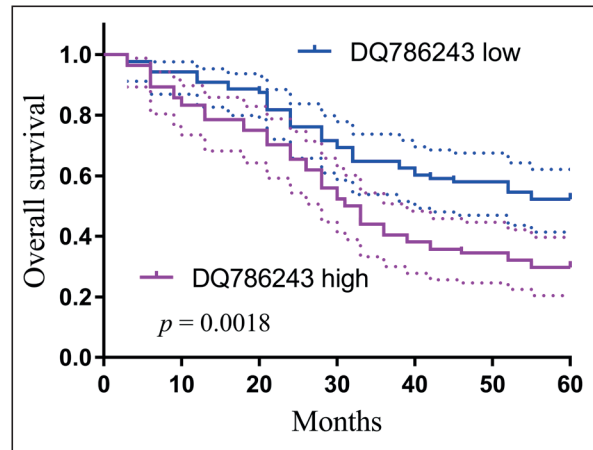
First, we measured the DQ786243 expression levels by RT-qPCR in a set of 172 matched samples. As shown in Figure 1, we found that the expression levels of DQ786243 in human GC tissues were significantly increased than in the paired normal tissues (*p* < 0.001). These data indicated that DQ786243 may be involved in the progression of GC.

**Association Between DQ786243 Expression Levels and Clinicopathological Characteristics**

To evaluate the correlation between DQ786243 expression and clinicopathological



**Figure 1.** RT-PCR was performed to identify the relative expression of DQ786243 in 172 GC tissues. The expression levels of DQ786243 in GC tissues were significantly higher than those in corresponding noncancerous gastric tissues (*p* < 0.01).

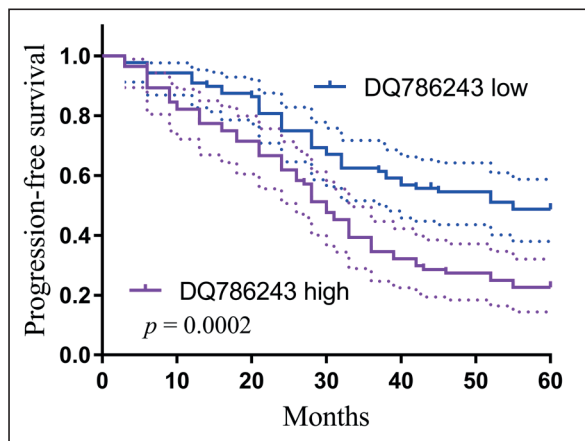


**Figure 2.** Kaplan-Meier curves for OS in patients with GC divided according to DQ786243 expression. Patients with higher levels of DQ786243 expression showed shorter OS times compared to patients with lower levels of DQ786243 expression (*p* = 0.0018).

characteristics, all 172 GC patients were divided into two subgroups according to mean value: a high-DQ786243 group (84 cases) and a low miR-DQ786243 group (88 cases) based on the median (6.65) DQ786243 level. Then,  $\chi^2$ -tests were performed. High expression of DQ786243 was found to significantly correlate with higher invasion depth (*p* = 0.006), TNM stage (*p* = 0.009) and lymphatic metastasis (*p* = 0.017) (Table I). However, several other clinical parameters were found not to be significantly associated with DQ786243 expression in this study.

**Correlation Between DQ786243 Expression and Prognosis of GC Patients**

To investigate the influence of the expressions of DQ786243 on overall survival (OS) and progression-free survival (PFS), Kaplan-Meier survival curves were constructed. It was found that patients with high DQ786243 expression showed poorer OS (Figure 2, *p* = 0.0012) and PFS (Figure 3, *p* = 0.002) compared with the patients with low DQ786243 expression. Next, we used multivariate Cox regression analysis to evaluate the prognostic value of DQ786243. As shown in Table II, we found that DQ786243 was an independent prognostic factor for OS (HR 3.381, 95% CI 1.572-7.732, *p* = 0.002) and PFS (HR 3.667, 95% CI 1.783-8.933, *p* = 0.001), as well as invasion depth, TNM stage and lymphatic metastasis (all *p* < 0.05).



**Figure 3.** Kaplan-Meier curves for PFS in patients with GC divided according to DQ786243 expression. Patients with higher levels of DQ786243 expression showed shorter OS times compared to patients with lower levels of DQ786243 expression ( $p = 0.0002$ ).

## Discussion

In China, GC is the primary public health problem, which results in a lot of casualties and economic losses<sup>19</sup>. One of the most important ways for decreasing the mortality rate and improving the prognosis for GC patients is the early detection of this disease<sup>20</sup>. However, the sensitivity and specificity of the current diagnostic method are low. The discovery of lncRNAs roles in cell function has opened up possible applications in exploring useful markers for clinical outcome and diagnosis for GC<sup>21,22</sup>. In addition, clinical practice indicates that effective biomarkers are very important for doctors to choose appropriate therapies. Therefore, the identification of novel diagnostic and prognostic biomarkers is rather significant, and our attention focuses on lncRNAs.

In the past decades, growing evidence has reported that lncRNAs were implicated in a wide range of biological functions, and more and more lncRNAs were identified in GC. For instance, Du et al<sup>23</sup> reported that down-regulation of lncRNA WT1-AS was found in GC patients, and its forced expression suppressed the proliferation and invasion of GC cells. Chen et al<sup>24</sup> found that lncRNA XIST was activated in gastric cancer and promotes cell proliferation and metastasis by acting as a molecular sponge of miR-101. Wang et al<sup>25</sup> showed that lncRNA BCAR4 was highly expressed in GC tissues, and its high expression was involved in cisplatin resistance. These results showed the great value of lncRNA in regulation GC progression. A novel lncRNA, DQ786243, was reported to be implicated in oncogenesis. Sun et al<sup>17</sup> showed that DQ786243 served as a tumor promoter in colorectal cancer by promoting proliferation and metastasis of colorectal cancer. Shun et al<sup>18</sup> also indicated the tumor-promotive role of DQ786243 in GC. Of note, their clinical assay revealed that increased DQ786243 expression was positively associated with advanced clinicopathological features of GC patients. Based on these results, further study of clinical significance of DQ786243 was needed.

We found that DQ786243 was significantly overexpressed in GC tissues, which was consistent with the previous results. More importantly, DQ786243 expression correlated with invasion depth, TNM stage, and lymphatic metastasis, suggesting that DQ786243 act as an important role in the clinical progression of GC patients. Furthermore, the data of Kaplan-Meier analyses revealed that GC tissues, with the increased expression of DQ786243, tend to have shorter OS and PFS. In addition, the prognostic value of DQ786243 was further demonstrated by the Cox

**Table II.** Multivariate Cox proportional hazard model analysis of overall survival and progression-free survival in GC patients

	Overall survival			Progression-free survival		
	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
Age	1.432	0.782-1.884	0.217	1.213	0.664-1.732	0.328
Gender	0.933	0.417-2.138	0.318	0.774	0.317-2.336	0.581
Differentiation	1.325	0.782-2.556	0.114	1.563	0.631-2.773	0.097
Lauren type	1.673	0.642-3.421	0.139	1.427	0.885-2.993	0.177
Tumor size	1.832	0.885-2.935	0.193	1.428	0.563-2.238	0.115
Invasion depth	3.554	1.347-7.783	0.006	4.232	1.673-8.845	0.003
TNM stage	3.231	1.557-5.563	0.008	3.672	1.732-6.532	0.004
Lymphatic metastasis	4.231	1.321-8.563	0.009	3.563	1.556-6.674	0.013
DQ786243 expression	3.381	1.572-7.732	0.002	3.667	1.783-8.933	0.001

proportional hazard model. The results suggested that high expression level of DQ786243 was an independent poor prognosis factor for GC. To our knowledge, this is the first report determining the prognostic value of DQ786243 by Kaplan-Meier survival assay and multivariate analysis.

## Conclusions

We showed that DQ786243 overexpression is associated with a poor prognosis in patients with GC. Therefore, DQ786243 could be a potential independent prognostic factor, but further studies are required to reveal the role of DQ786243 in GC.

## Conflict of Interest

The Authors declare that they have no conflict of interests.

## References

- 1) FERLAY J, SHIN HR, BRAY F, FORMAN D, MATHERS C, PARKIN DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; 127: 2893-2917.
- 2) TORRE LA, BRAY F, SIEGEL RL, FERLAY J, LORTET-TIEULENT J, JEMAL A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; 65: 87-108.
- 3) VALENZUELA MA, CANALES J, CORVALÁN AH, QUEST AF. Helicobacter pylori-induced inflammation and epigenetic changes during gastric carcinogenesis. *World J Gastroenterol* 2015; 21: 12742-12756.
- 4) DULAK AM, SCHUMACHER SE, VAN LIESHOUT J, IMAMURA Y, FOX C, SHIM B, RAMOS AH, SAKSENA G, BACA SC, BASELGA J, TABERNERO J, BARRETINA J, ENZINGER PC, CORSO G, ROVIELLO F, LIN L, BANDLA S, LUKETICH JD, PENNATHUR A, MEYERSON M, OGINO S, SHIVDASANI RA, BEER DG, GODFREY TE, BEROUKHIM R, BASS AJ. Gastrointestinal adenocarcinomas of the esophagus, stomach, and colon exhibit distinct patterns of genome instability and oncogenesis. *Cancer Res* 2012; 72: 4383-4393.
- 5) YOUNG JA, SHIMI SM, KERR L, McPHILLIPS G, THOMPSON AM. Reduction in gastric cancer surgical mortality over 10 years: an adverse events analysis. *Ann Med Surg (Lond)* 2014; 3: 26-30.
- 6) HARTGRINK HH, JANSEN EP, VAN GRIEKEN NC, VAN DE VELDE CJ. Gastric cancer. *Lancet* 2009; 374: 477-490.
- 7) FATICA A, BOZZONI I. Long non-coding RNAs: new players in cell differentiation and development. *Nat Rev Genet* 2014; 15: 7-21.
- 8) GIBB EA, BROWN CJ, LAM WL. The functional role of long non-coding RNA in human carcinomas. *Mol Cancer* 2011; 10: 38.
- 9) SCHMITT AM, GARCIA JT, HUNG T, FLYNN RA, SHEN Y, QU K, PAYUMO AY, PERES-DA-SILVA A, BROZ DK, BAUM R, GUO S, CHEN JK, ATTARDI LD, CHANG HY. An inducible long noncoding RNA amplifies DNA damage signaling. *Nat Genet* 2016; 48: 1370-1376.
- 10) KHORSHIDI A, DHALIWAL P, YANG BB. Noncoding RNAs in tumor angiogenesis. *Adv Exp Med Biol* 2016; 927: 217-241.
- 11) LI DS, ANIWAER JL, SHEYHIDING I, ZHANG Z, ZHANG LW. Identification of key long non-coding RNAs as competing endogenous RNAs for miRNA-mRNA in lung adenocarcinoma. *Eur Rev Med Pharmacol Sci* 2016; 20: 2285-2295.
- 12) FU JW, KONG Y, SUN X. Long noncoding RNA NEAT1 is an unfavorable prognostic factor and regulates migration and invasion in gastric cancer. *J Cancer Res Clin Oncol* 2016; 142: 1571-1579.
- 13) QI P, XU MD, SHEN XH, NI SJ, HUANG D, TAN C, WENG WW, SHENG WO, ZHOU XY, DU X. Reciprocal repression between TUSC7 and miR-23b in gastric cancer. *Int J Cancer* 2015; 137: 1269-1278.
- 14) FENG Y, ZHANG Q, WANG J, LIU P. Increased lncRNA AFAP1-AS1 expression predicts poor prognosis and promotes malignant phenotypes in gastric cancer. *Eur Rev Med Pharmacol Sci* 2017; 21: 3842-3849.
- 15) YANG F, ZHANG L, HUO XS, YUAN JH, XU D, YUAN SX, ZHU N, ZHOU WP, YANG GS, WANG YZ, SHANG JL, GAO CF, ZHANG FR, WANG F, SUN SH. Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. *Hepatology* 2011; 54: 1679-1689.
- 16) QIAO YQ, HUANG ML, XU AT, ZHAO D, RAN ZH, SHEN J. LncRNA DQ786243 affects Treg related CREB and Foxp3 expression in Crohn's disease. *J Biomed Sci* 2013; 20: 87.
- 17) SUN L, XUE H, JIANG C, ZHOU H, GU L, LIU Y, XU C, XU O. LncRNA DQ786243 contributes to proliferation and metastasis of colorectal cancer both in vitro and in vivo. *Biosci Rep* 2016; 36: pii: e00328.
- 18) SHAN T, FAN J, ZHAO Q, DENG K, XIA J. Upregulation of long non-coding RNA DQ786243 promotes the progression of gastric cancer. *Mol Med Rep* 2017; 16: 3761-3768.
- 19) STRONG VE, WU AW, SELBY LV, GONEN M, HSU M, SONG KY, PARK CH, COIT DG, JI JF, BRENNAN MF. Differences in gastric cancer survival between the U.S. and China. *J Surg Oncol* 2015; 112: 31-37.
- 20) PASECHNIKOV V, CHUKOV S, FEDOROV E, KIKUSTE I, LEJA M. Gastric cancer: prevention, screening and early diagnosis. *World J Gastroenterol* 2014; 20: 13842-13862.
- 21) VAN ROOSBROECK K, POLLET J, CALIN GA. miRNAs and long noncoding RNAs as biomarkers in human diseases. *Expert Rev Mol Diagn* 2013; 13: 183-204.
- 22) ZHOU X, YIN C, DANG Y, YE F, ZHANG G. Identification of the long non-coding RNA H19 in plasma as a

- novel biomarker for diagnosis of gastric cancer. *Sci Rep* 2015; 5: 11516.
- 23) DU T, ZHANG B, ZHANG S, JIANG X, ZHENG P, LI J, YAN M, ZHU Z, LIU B. Decreased expression of long non-coding RNA WT1-AS promotes cell proliferation and invasion in gastric cancer. *Biochim Biophys Acta* 2016; 1862: 12-19.
- 24) CHEN DL, JU HO, LU YX, CHEN LZ, ZENG ZL, ZHANG DS, LUO HY, WANG F, QIU MZ, WANG DS, XU DZ, ZHOU ZW, PELICANO H, HUANG P, XIE D, WANG FH, LI YH, XU RH. Long non-coding RNA XIST regulates gastric cancer progression by acting as a molecular sponge of miR-101 to modulate EZH2 expression. *J Exp Clin Cancer Res* 2016; 35: 142.
- 25) WANG L, CHUNYAN Q, ZHOU Y, HE Q, MA Y, GA Y, WANG X. BCAR4 increase cisplatin resistance and predicted poor survival in gastric cancer patients. *Eur Rev Med Pharmacol Sci* 2017; 21: 4064-4070.