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ORIGINAL ARTICLE

Influences of lncRNA HEIH and DKK3 on the clinical features and prognosis of gastric cancer

Hui Dong, Fengjie Li, Aichun Jin

Emergency Internal Medicine, LuHe Hospital of Capital Medical University, Beijing, China.

Summary

Purpose: *To detect differential expressions of HEIH and DKK3 in gastric cancer (GC) samples, and to elucidate their influences on clinical features and disease prognosis.*

Methods: *The expression levels of HEIH and DKK3 in GC tissues and adjacent normal ones (>5 cm) were detected by qRT-PCR. Correlation between HEIH and DKK3 levels in GC tissues was analyzed by Pearson's correlation analysis. Sensitivity and specificity of detecting HEIH and DKK3 levels in diagnosing GC were assessed by reactive oxygen species (ROC). 5-year survival in each patient was followed up. Risk factors of prognosis in GC patients were examined by Cox regression model.*

Results: *HEIH was upregulated, and DKK3 was downregulated in GC tissues, displaying a negative correlation. Both HEIH and DKK3 were correlated to tumor diameter, lymph node metastasis and TNM staging. Combined detection of HEIH and DKK3 levels showed high specificity and sensitivity in the diagnosis of GC. Tumor diameter, lymph node metastasis, TNM staging, HEIH and DKK3 levels were independent risk factors for the prognosis of GC.*

Conclusion: *The upregulated HEIH and downregulated DKK3 in GC samples showed a negative correlation between each other. HEIH and DKK3 levels were closely linked to tumor diameter, lymph node metastasis and TNM staging in GC patients. These are promising biomarkers for predicting the prognosis of GC.*

Key words: *gastric cancer, LncRNA HEIH, DKK3, prognosis, biomarker*

Introduction

Gastric cancer (GC) is highly prevalent in digestive system tumors. Its pathogenesis is complicated, and atypical early-stage symptoms are easily to be neglected, thus leading to a low detect on rate of GC in the early phase [1,2]. At present, radical gastrectomy is an effective therapeutic strategy for GC patients in the early-stage. However, most of middle stage or advanced GC patients cannot be operated, and they suffer a poor prognosis [3]. Searching for specific biomarkers is conductive to improve clinical outcomes of GC.

In eukaryotes, the number of non-coding RNAs far exceeds that of protein-encoding genes. Long non-coding RNAs (LncRNAs) are exceeding 200 nucleotides in transcripts. They lack open reading frame to encode proteins [4,5]. During the progression of GC, multiple abnormally expressed lncRNAs have been discovered. They have a close relation to malignant phenotypes of tumor cells and tumor progression [6,7]. LncRNA HEIH was firstly reported in hepatocellular carcinoma profiling, which aggravated tumor progression by mediating cell cycle progression and displaying a prognostic potential [8]. Later, HEIH has been identified to be upregulated in many other tumors (i.e. colorectal carcinoma, non-small-cell lung carcinoma, melanoma), and serves as a prognostic factor [9-11].

Corresponding author: Fengjie Li, MM. Emergency Internal Medicine, LuHe Hospital of Capital Medical University, No. 82 of Xinhua South Street, Tongzhou, Beijing 101149, China.
Tel: +86 013611115537; Email: 13611115537@163.com
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The DKK (Dickkopf) family contains four members, i.e. DKK1, DKK2, DKK3 and DKK4 [12]. DKK1/2/4 are mainly involved in the regulation of the Wnt signaling. DKK3 is considered as a vital regulator involved in tumor progression [13]. It is reported that DKK3 is downregulated in many types of tumors. Overexpression of DKK3 can inhibit the proliferative potential and induce apoptosis in tumor cells [14,15]. A recent study has shown that lncRNA SNHG16 triggers epidermal-mesenchymal transition (EMT) in GC *via* downregulating DKK3 [16].

In this study, we first detected the expression levels of HEIH and DKK3 in GC tissues. Subsequently, their clinical significances in affecting the prognosis of GC were mainly illustrated.

Methods

Subjects and samples

GC tissues and adjacent normal ones were collected from 150 eligible patients who did not have any anticancer treatment before surgery. Tissue samples were quickly frozen in liquid nitrogen and stored at -80°C for use. Medical staff responsible for collecting samples and labeling did not participate in the later experiments. This study got approval of Ethics Committee of our Hospital. All subjects signed the written informed consent.

Clinical diagnosis and pathological parameters of GC patients were independently confirmed by two experienced pathologists. TNM staging was defined according to the 8th edition proposed by the American Joint Committee on Cancer [17]. Histological subtypes of GC were defined based on the 2010 WHO classification of tumors of digestive system [18]. Overall survival (OS) was the time duration from surgery to death.

qRT-PCR

Total RNAs in tissues were collected using the RNAiso Plus (TaKaRa, Dalian, China) and reversely transcribed to complementary DNAs (cDNAs). A reaction mixture (20 μL) containing 400 ng cDNA, 10 μL of TB Green series (TaKaRa, Dalian, China), 0.6 μL of forward sequence, 0.6 μL of reverse sequence and 6.8 μL of RNase-free ddH_2O was subjected to qRT-PCR at 95 oC for 15 min, and 40 cycles at 95°C for 10 s, 56°C for 20 s and 72 for 30 s. Primers were synthesized using Primer Blast as follows: HEIH: 5'-CCTCTTGTGCCCCTTTCT-3' (forward) and 5'-AGGTCTCATGGCTTCTCG-3' (reverse); DKK3: 5'-ACAGCCACAGCCTGGTGTA-3' (forward) and 5'-CCTCCATGAAGCTGCCAAC-3' (reverse);GAPDH: 5'-TGTTGCCATCAATGACCCCTT-3' (forward) and 5'-CTCCACGACGTACTCAGCG-3'.

Statistics

SPSS 22.0 software was used for statistical analyses. Data was expressed as mean ± SD. Measurement data and enumeration data were compared using the t-test and x^2 test, respectively. Pearson's correlation analysis was conducted to assess the correlation between HEIH and DKK3 levels and their diagnostic potential was evaluated by plotting ROC curves. Risk factors of prognosis in GC patients were examined by Cox regression model. P<0.05 showed statistical significance.

Results

Expression levels of HEIH and DKK3 in GC

Compared with paracancer tissues, HEIH was upregulated and DKK3 was downregulated in GC tissues (Figure 1A,1B), showing possible involvement in the progression of GC.

Influence of HEIH and DKK3 on clinical features in GC

Based on the median level of HEIH in GC tissues, patients were classified to a low level group (n=75) and a high level group (n=75). No significant differences in sex, age and histological subtypes were identified between groups (p>0.05). However, significant differences in tumor diameter, TNM staging and nodal metastasis status were observed

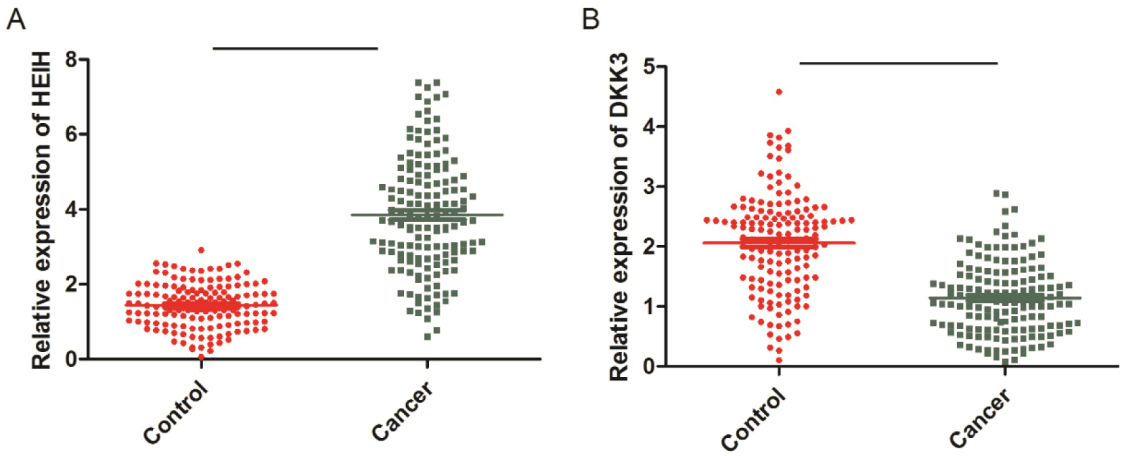

Figure 1. Expression levels of HEIH **(A)** and DKK3 **(B)** in gastric cancer and paracancer tissues (*p<0.05).

(p<0.05) (Table 1). In the same way, patients were classified into two groups according to the median level of DKK3. Significant differences were detected in tumor diameter, TNM staging and nodal metastasis status (p<0.05) (Table 2).

Correlation between HEIH and DKK3 levels in GC

Pearson's correlation analysis revealed a negative correlation between HEIH and DKK3 levels in GC tissues (r=-0.7859, p<0.001) (Figure 2).

Diagnostic potential of HEIH and DKK3 in GC

To elucidate diagnostic potential of HEIH and DKK3 in GC, ROC curves were plotted using the follow-up data we recorded. The sensitivity and specificity of detecting HEIH level in diag-

Table 1. Correlation between HEIH and clinical features in gastric cancer

Variables	*n*	*Low level (n=75)*	*High level (n=75)*	x^2	*p*
Sex					
Male	78	35	43	1.709	0.253
Female	72	40	32		
Age, years					
<60	71	39	32	1.310	0.327
≥60	79	36	43		
Histological subtype					
Adenocarcinoma	61	28	33	0.691	0.506
Squamous cell carcinoma	89	47	42		
Tumor diameter (cm)					
≤5	67	41	26	6.069	0.021
>5	83	34	49		
TNM staging					
I+II	77	45	27	8.654	0.005
III+IV	73	30	48		
Lymph node metastasis					
No	74	44	30	5.228	0.033
Yes	76	31	45		

Table 2. Correlation between DKK3 and clinical features in gastric cancer patients (n=150)

Variables	*n*	*Low level (n=75)*	*High level (n=75)*	x^2	*p*
Sex					
Male	78	36	42	0.962	0.414
Female	72	39	33		
Age, years					
<60	71	37	34	0.241	0.744
≥60	79	38	41		
Histological subtype					
Adenocarcinoma	61	30	31	0.028	1.000
Squamous cell carcinoma	89	45	44		
Tumor diameter (cm)					
≤5	67	24	43	9.737	0.003
>5	83	51	32		
TNM staging					
I+II	77	29	48	9.634	0.003
III+IV	73	46	27		
Lymph node metastasis					
No	74	30	44	5.228	0.033
Yes	76	45	31		

nosing GC were 78.00% and 81.20%, respectively (Area Under the Curve/AUC=0.784, Youden index=0.592). Detection of DKK3 was also capable of sensitively and specifically diagnosing GC (sensitivity=80.40%, specificity=83.57%, AUC=0.823, Youden index=0.64). Notably, combined detection of HEIH and DKK3 showed the largest AUC (0.937), showing a promising prognostic potential in GC (sensitivity=90.51%, specificity=89.9%, Youden index=0.804) (Table 3).

Risk factors for the prognosis of GC

Based on the above findings, significant variables, including tumor diameter, TNM staging, lymph node metastasis status, HEIH level and DKK3 level were subjected to the Cox regression analysis which showed tumor diameter ≥5 cm, occurrence of nodal metastasis, stage III+IV and high level of HEIH were independent risk factors for the prognosis of OS, whereas DKK3 was the protective factor (Table 4).

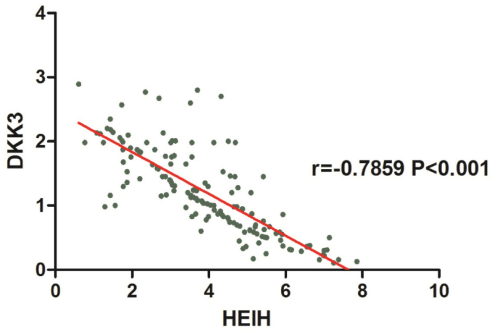

Figure 2. A negative correlation between DKK3 and HEIH levels in gastric cancer tissues (r=-0.7859, p<0.001).

Discussion

Although great efforts have been made on improving therapeutic strategies, the mortality of GC remains fifth in the world [19]. Diagnostic markers for GC in the early phase are lacking, and its pathogenesis is largely unknown. About 80% of GC patients are diagnosed in middle or advanced stage, and their 5-year OS is only 25% [20]. It is necessary to seek for new diagnostic biomarkers and therapeutic targets of GC.

LncRNAs are involved in the regulation of chromosome silencing, genomic imprinting, transcriptional activity and other life activities by interacting with proteins, DNAs or RNAs [21]. Dysfunctional lncRNAs are closely linked to malignant phenotypes of tumor cells. It is reported that lncRNA H19 is upregulated in GC patients and responsible for driving tumor cell proliferation [22]. Sun et al [23] demonstrated that lncRNA MEG3 is lowly expressed in GC tissues. Knockdown of MEG3 markedly induces *in vitro* growth of GC cells. LncRNAs are seen as promising therapeutic targets for anticancer treatment [24]. In addition, HEIH has been previously reported to deteriorate

Table 3. Sensitivity and specificity of HEIH and DKK3 in diagnosing gastric cancer

Variables	*AUC*	*p*	*Sensitivity %*	*Specificity %*	*Youden Index*
HEIH	0.784	0.031	78.00	81.20	0.592
DKK3	0.823	0.016	80.40	83.57	0.64
HEIH+DKK3	0.937	0.002	90.51	89.93	0.804

Table 4. Cox regression analysis on potential factors influencing the overall survival in gastric cancer

Variables	*HR*	*95%CI*	*p*
Tumor diameter (cm)			
(≤5, >5)	1.488	1.215-2.752	0.004
TNM staging			
(I+II, III+IV)	1.983	1.375-3.775	0.031
Lymph node metastasis			
(No, Yes)	1.598	1.224-4.315	0.017
HEIH level			
(Low, High)	2.315	1.875-4.352	0.008
DKK3 level			
(Low, High)	0.586	0.335-0.874	<0.001

HR=hazard ratios, CI=confidence interval

the progression of breast cancer *via* activating the Wnt signaling through targeting miR-200b [25]. Compared with paracancer tissues, HEIH is upregulated in hepatocellular carcinoma tissues. It is capable of regulating MMPs, cell cycle proteins and apoptosis-associated genes, thus influencing cancer progression [26]. Our findings showed that HEIH was upregulated in GC tissues, which was unfavorable to the prognosis of GC as an independent risk factor.

DKK3 is a newly detected tumor suppressor gene, which is able to affect the Wnt signaling through competitively binding some components [27]. The biological functions of DKK3 in tumor cell behaviors have been identified. For example, overexpression of DKK3 stimulates apoptosis of lung cancer cells and suppresses their proliferative and invasive capabilities [28]. The growth of pancreatic cancer is slowed down and cell apoptosis is triggered by overexpression of DKK3 [29]. DKK3 consistently exerts an anticancer effect on colorectal carcinoma [30]. Huang et al [31] demonstrated that MEG3 induces lipogenesis and angiogenesis in the preadipocyte cell line 3T3-L1 by activating the VEGF signaling and inhibiting the Wnt signaling with the involvement of DKK3. A previous study reported that DKK3 is downregulated in GC tissues and SNHG16 is upregulated, displaying a negative correlation [16]. Our findings consistently showed that DKK3 was downregulated in GC tissues, presenting a negative correlation to HEIH level. Highly expressed DKK3 was a protective factor for the prognosis of GC and we believe that HEIH and DKK3 are promising biomarkers for the diagnosis and treatment of GC.

Conclusions

The upregulated HEIH and downregulated DKK3 in GC samples showed a negative correlation between each other. HEIH and DKK3 levels are closely linked to tumor diameter, nodal metastasis and TNM staging in GC patients and they are promising biomarkers for predicting the prognosis of GC.

Conflict of interests

The authors declare no conflict of interests.

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