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Original article

Increased expression of LncRNA PANDAR predicts a poor prognosis in gastric cancer

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ABSTRACT

Long non-coding RNAs (lncRNAs) are emerging as biomarkers and as important regulators in biological processes and tumorigenesis in cancer. PANDAR (promoter of CDKN1A antisense DNA damage activated RNA) serves as biomarkers and involves in development of multiple cancers. However, its clinical value of PANDAR in gastric cancer is still unknown. Hence, we carried out the present study aiming to identify the clinical significance of PANDAR in gastric cancer patients. We analyzed the expression levels of PANDAR in 100 paired gastric cancer tissues using Quantitative Real-time PCR. Our results showed that the expression of PANDAR was significantly increased in gastric cancer tissues compared with paired adjacent normal tissues. Furthermore, high expression of PANDAR was correlated with depth of invasion, TNM stage and lymphatic metastasis. Importantly, high expression of PANDAR could serve as an independent unfavorable prognostic role in gastric cancer. In conclusion, PANDAR may be a potential novel biomarker that predicts prognosis in gastric cancer.

1. Introduction

Gastric cancer is the fourth most frequently diagnosed cancer and its mortality ranks the third in cancer in the world [1]. About 7.2 million gastric cancer-related deaths occurred worldwide in 2012, particularly in East Asia [1]. Because most patients are diagnosed at advanced stage, the prognosis of unresectable or metastatic gastric cancer patients remains unsatisfactory [2]. It is known that gastric cancer is curable if detected early, so it is urgent to find novel biomarkers for diagnosis and prognosis evaluation.

Long noncoding RNAs (lncRNAs), greater than 200 nucleotides (nts) in length, have attracted great attention in the past few years. LncRNAs are important members of ncRNA family without the capacity of coding proteins. A mountain number of studies have revealed that lncRNAs play important roles in regulating proliferation, apoptosis, invasion, metastasis and other biological processes [3–8]. Functional lncRNAs can be applied for cancer diagnosis and prognosis, and also could be potential therapeutic targets [9].

LncRNA PANDAR (promoter of CDKN1A antisense DNA damage activated RNA) is 1506 nts in length and located at chr6p21.2, which we focus on in this study was firstly reported by Hung and Wang [10]. They found PANDAR is induced by a p53-depentent manner interacts with transcription factor NF-YA, which is the regulatory subunit of nuclear transcription factor Y (NF-Y), to limit the expression of pro-apoptotic genes in human fibroblasts [10] NF-YA is reported to correlate with clinical prognosis in multiple cancers, and involves in tumorgenesis impacting proliferation and apoptosis [11–14]. Lately, Han and Zhang, and Peng and Fan [15,16] showed ectopic expression of PANDAR could predict prognosis of non-small cell lung cancer and hepatocellular carcinoma respectively. The clinical relevance and the role in carcinogenesis of PANDAR in gastric cancer remain to be elucidated.

In the present study, we found that PANDAR was up-regulated in gastric cancer tissues compared with paired adjacent normal tissues. High expression of PANDAR was associated with clinicopathological characteristics and poor prognosis in gastric cancer.

2. Materials and methods

2.1. Tissue samples and clinical data collection

In this study, we collected 100 paired noncancerous and cancer tissue samples at the First Affiliated Hospital of Nanjing Medical University. The study was approved by the Ethics Committee on

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Human Research of the First Affiliated Hospital of Nanjing Medical University. All patients gave written informed consent. The clinical characteristics of the patients with gastric cancer were collected from their clinicopathological reports. The clinical follow-up time of patients ranged from 2 to 36 months. Physical examination, laboratory analysis and computed tomography if necessary were included in follow-up studies. Overall survival (OS) was defined as the interval between the dates of surgery and death. Disease-free survival (DFS) was defined as the interval between the dates of surgery and recurrence; if recurrence was not diagnosed, patients were censored on the date of death or the last follow-up.

2.2. RNA preparation and quantitative Real-time PCR

Total RNAs were extracted form cancerous and paired adjacent noncancerous tissues using Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacture's protocol. The isolated total RNA was reversing transcribed using PrimeScirpt RT Master Mix (Takara, Dalian, China) according to the manufacture's protocol. The sequence-specific forward and reverse primers for PANDAR were 5′-TGCACACATTTAACCCGAAG-3′ and 5′-CCCCAAAGCTACATC-TATGACA-3′ respectively. The forward and reverse primers for GAPDH were 5′-AGCCACATCGCTCAGACAC-3′ and 5′-GCCCAATAC-GACCAAATCC-3′ respectively. Quantitative real-time PCR (qPCR) was performed using SYBR Premix EX TaqTM II (Takara, Dalian, China) on 7900HT Fast Real-time System (Applied Biosystems, Forset City, CA, USA). All qPCR reactions were performed in triplicate and the relative expression of PANDAR was calculated using the comparative cycle threshold (CT) ($2^{-\Delta\Delta CT}$) method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous control to normalize the data.

2.3. Statistical analysis

All statistical analyses were performed using SPSS version 22.0 (IBM, Chicago, IL, USA) and GraphPad Prism 6 (GraphPad software, La Jolla, CA, USA). The significance of differences between groups was estimated by the χ2-test or Wilcoxon test, as appropriate. OS and DFS rates were calculated by the Kaplan–Meier method with the log-rank test applied for comparison. Survival data were evaluated using unilabiate and multivariate Cox regression methods. Variables with a value of $p < 0.05$ in the univariate analysis were used in subsequent multivariate analysis on the basis of Cox regression analyses. Two-sided p-value were calculated, and a probability level of 0.05 was chosen for statistical significance.

3. Results

3.1. PANDAR expression is up-regulated in gastric cancer tissues compared with paired adjacent normal tissues

PANDAR expression levels were detected in 100 paired fresh gastric cancer samples and adjacent normal tissues by quantitative polymerase chain reaction assays. PANDAR expression was significantly up-regulated in tumor tissues compared with the paired adjacent normal tissues ($p < 0.001$; Fig. 1).

3.2. PANDAR expression and clinic pathologic features in gastric cancer

In order to assess the correlation of PANDAR expression with clinicopathological features, the expression levels of PANDAR in tumor tissues were categorized as high or low compared with the corresponding adjacent noncancerous tissue samples. As shown in Table 1, the high PANDAR group ($n = 73$) showed a greater depth of invasion ($p < 0.001$), higher TNM stage ($p = 0.011$) and more frequent lymphatic metastasis ($p = 0.017$) than the low PANDAR group ($n = 27$). However, there was no significant correlation between PANDAR expression and other clinicopathological characteristics such as age, gender, tumor size, histologic differentiation and distant metastasis ($p > 0.05$).

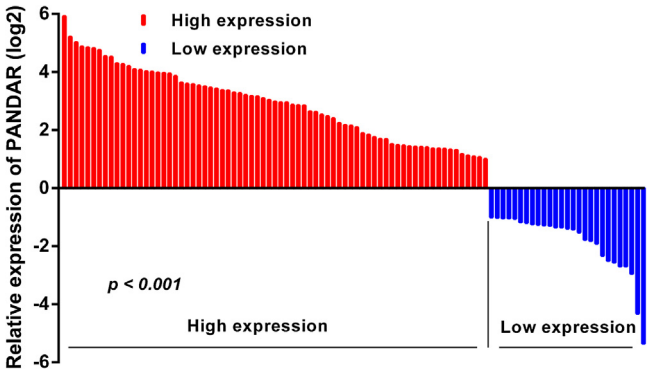

Fig. 1. The expression leves of PANDAR in gastric cancer tissues. PANDAR expression was significantly up-regulated in tumor tissues compared with the paired adjacent normal tissues ($p < 0.001$).

3.3. PANDAR expression is negatively associated with prognosis of patients with gastric cancer

We used Kaplan-Meier analysis and log-rank test to investigate the effects of PANDAR expression and the clinicopathological

Table 1
Correlation between PANDAR expression and clinicopathological characteristics of gastric cancer.

Clinical parameter	PANDAR		χ^2-test *P*-value
	High expression ($n = 73$)	Low expression ($n = 27$)	
Age			0.986
<50 years	35	13	
≥50 years	38	14	
Gender			0.541
Male	41	17	
Female	32	10	
Tumor size			0.889
< 5 cm	39	14	
≥ 5 cm	34	13	
Location			0.904
Cardia + body	45	17	
Pylorus	28	10	
Histologic differentiation			0.408
Well + moderate	48	20	
Poor + undifferentiated	25	7	
Depth of invasion			<0.001[a]
T1 + T2	27	22	
T3 + T4	46	5	
TNM stage			0.011[a]
I + II	39	22	
III + IV	34	5	
Lymphatic metastasis			0.017[a]
NO	15	12	
YES	58	15	
Distant metastasis			0.557
NO	68	26	
YES	5	1	

[a] Overall $P < 0.05$.

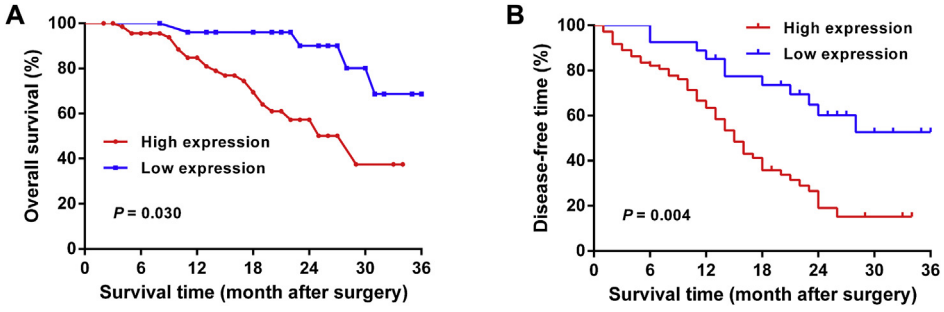

Fig. 2. OS and DFS of gastric cancer patients with higher PANDAR expression levels compared to low PANDAR expression levels.
A). The patients in the high PANDAR group had a shorter overall survival (median OS: 24.4 months) than those in the low PANDAR group (median OS: 32.9 months; $p = 0.030$).
B). The patients in the high PANDAR group had a higher recurrence rate (median DFS: 16.6 months) than those in the low PANDAR group (median DFS: 27.2 months; $p = 0.004$).

factors on overall survival (OS) and disease-free survival (DFS). The patients in the high PANDAR group had a shorter overall survival (median OS: 24.4 months) and a higher recurrence rate (median DFS: 16.6 months) than those in the low PANDAR group (median OS: 32.9 months; median DFS: 27.2 months; $p = 0.030$ and 0.004, respectively; Fig. 2). The univariate Cox regression analyses of OS revealed PANDAR expression ($p = 0.006$), depth of invasion ($p = 0.027$), TNM stage ($p < 0.001$) and lymphatic metastasis ($p = 0.024$) were prognostic indicators, and the univariate Cox regression of DFS revealed PANDAR expression ($p = 0.001$), depth of invasion ($p = 0.012$), TNM stage ($p < 0.001$), lymphatic metastasis ($p = 0.001$) and distant metastasis ($p = 0.029$) were prognostic indicators. Furthermore, the multivariate Cox regression analyses of OS and DFS both suggested the PANDAR expression was an independent prognostic indicator for OS (hazard ratio [HR] = 3.683; 95% confidence interval [CI], 1.125–12.058; $p = 0.031$) and DFS (HR = 2.359; 95% CI, 1.153–4.830; $p = 0.019$) in patients with gastric cancer (Table 2).

4. Discussion

Gastric cancer is the third lethal cause of cancer-related death worldwide, and its morbidity remains high especially in East Asia [1]. Although prognosis of gastric cancer has been improved greatly by target therapy such as Her2 inhabitors, PD-1/PDL-1 checkpoint inhibitors and VEGF inhibitors, the prognosis is hardly satisfactory and treatment outcome primarily depends on early detection [17,18]. Therefore, identification and use of molecular biomarkers in early detection and personalization of therapy should improve patients outcome [18]. However, identification of molecular biomarkers with clinical value is an enormous challenge.

Coding-protein genes only account for 2%, the majority of the human genome is composed of non-coding RNAs, suggesting that ncRNAs could play important roles in complex organisms [3]. Many lncRNAs have been implicated in cancer, such are microRNAs. MALAT-1 has been reported to enhance cell motility of lung adenocarcinoma [19]. Besides, MALAT-1 can serve as a molecular biomarkers for cancer metastasis and prognosis [20]. Zhang reveled that the higher expression of lncRNA ANDRIL predicts a poor prognosis and promotes tumor growth in gastric cancer [21]. However, biomarkers in gastric cancer remains largely unknown.

LncRNAs can modulate gene expression in diverse ways, including chromatin remodeling, transcriptional and post-transcriptional processing [22,23]. X inactive specific transcript (XIST) is a well-known lncRNA that plays an essential function in X chromosome inactivation (XCI) [24]. XIST RNA, from the inactive X chromosome, recruits the chromatin-modifying complex PRC2 to the transcription site, resulting in stable epigenetic silence of wide gene expression in X-chromosome during female development

Table 2
Univariate and multivariate Cox regression analyses PANDAR for OS and DFS of patients in study cohort ($n = 100$).

Variables	OS			DFS		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Univariate analysis						
Age (<50 years vs. ≥50 years)	0.549	0.244–1.235	0.147	0.820	0.488–1.375	0.451
Gender (male vs. female)	1.114	0.504–2.463	0.789	0.955	0.569–1.601	0.861
Tumor size (<5 cm vs. ≥5 cm)	0.887	0.410–1.920	0.761	0.775	0.465–1.293	0.330
Location (cardia + body vs. pylorus)	0.463	0.214–1.001	0.504	0.631	0.378–1.053	0.078
Histologic differentiation (well + moderate vs. poor + undifferentiated)	0.521	0.231–1.176	0.116	0.606	0.355–1.034	0.066
Depth of invasion (T1 + T2 vs. T3 + T4)	0.408	0.184–0.903	0.027[a]	0.514	0.305–0.866	0.012[a]
TNM stage (I + II vs. III + IV)	0.185	0.081–0.425	<0.001[a]	0.217	0.158–0.465	<0.001[a]
Lymphatic metastasis (NO vs. YES)	0.291	0.099–0.851	0.024[a]	0.296	0.145–0.606	0.001[a]
Distant metastasis (NO vs. YES)	0.349	0.079–1.541	0.165	0.354	0.139–0.901	0.029[a]
PANDAR expression (High vs. Low)	4.612	1.539–13.825	0.006[a]	3.113	1.591–6.093	0.001[a]
Multivariate analysis						
Depth of invasion (T1 + T2 vs. T3 + T4)	1.485	0.538–4.100	0.446	1.264	0.644–2.483	0.496
TNM stage (I + II vs. III + IV)	0.213	0.066–0.689	0.010[a]	0.373	0.176–0.788	0.010[a]
Lymphatic metastasis (NO vs. YES)	0.648	0.189–2.220	0.490	0.502	0.229–1.097	0.084
Distant metastasis (NO vs. YES)	–	–	–	0.768	0.290–2.029	0.768
PANDAR expression (High vs. Low)	3.683	1.125–12.058	0.031[a]	2.359	1.153–4.830	0.019[a]

Abbreviations: CI, confidence interval; DFS, disease-free survival; HR, hazard ratio.
[a] Overall $P < 0.05$.

[25]. Besides regulating chromatin modeling, lncRNAs can also influence the activity of specific transcription factors and polymerases [26]. The long polyadenylated Evf2 lncRNA is transcribed from an ultraconserved distal enhancer region associated with the Dlx-5/6 locus. It serves as a transcriptional coactivator by forming a complex with Dlx2 and then, specifically binding to the Dlx-5/6 enhancer to increase its transcriptional activity in a target and homeodomain-specific manner [27]. In post-transcriptional regulation, lncRNAs were reported to affect various post-transcriptional processes, including miRNA sponges and messenger RNA splicing, translation and stability. Wang and Liu [28] revealed that Highly Up-regulated in Liver Cancer (HULC) is able to inhibit the expression and activity of miR-372 by acting as an endogenous sponge, leading to translational depression of PRKCAB, and then PAKCAB induces the phosphorylation and activation of CREB, which allows lncRNA HULC to further increase its own expression levels. Xu and Liu [4] indicated that SP1 induced lncRNA TINCR recruits staufen 1 to the 3′UTR of the KLF2 mRNA, degrading KLF2 through the UPF1-dependent mRNA decay mechanism in gastric cancer.

PANDAR was firstly reported by Hung and Wang [10] who showed that PANDAR is induced in a p53-dependent manner and interacts with the transcription factor NF-YA to limit expression of pro-apoptotic genes; PANDAR depletion markedly sensitized human fibroblasts to apoptosis by doxorubicin. Peng and Fan [16] indicated low expression of PANDAR predicts a poor prognosis of NSCLC and PANDAR overexpression significantly repressed the proliferation partly due to the transcriptional modulation of Bcl-2 by interacting with NF-YA. NF-YA, the regulatory subunit of NF-Y transcription factor that binds to CCAAT sites, is reported to be engaged in multiple biological effects such as proliferation and apoptosis through controlling the expression of several key regulators of the cell cycle and apoptosis-related genes such as topoisomerase II *alpha*, CDK1, cyclins, cdc25c, Bcl-xl and Bcl2 [14,29–33].

To explore the role of PANDAR in gastric cancer, we used qPCR to find ectopic expression of PANDAR in gastric cancer. The results showed that PANDAR was frequently up-regulated in gastric cancer tissues than normal tissues. Then, we investigated the correlation between PANDAR and clinicopathological features. Moreover, patients with higher expression of PANDAR appeared to have a greater depth of invasion, an advanced TNM stage.

We also evaluated the relationship between PANDAR expression and prognosis of patients with gastric cancer. Kaplan–Meier analyses showed that patients with higher PANDAR expression tended to live a shorter life and have a relapse earlier than those with lower expression. Both univariate and multivariate COX regression analyses further revealed that the expression of PANDAR was an independent predictive factor of poor prognosis of patients with gastric cancer. These results suggested aberrant expression of PANDAR might involve in the biogenesis and development of gastric cancer. To date, our study is the first report showed that PANDAR is up-regulated in gastric cancer and significantly influences prognosis of gastric cancer patients, and may be an important predictor of outcome of patients.

5. Conclusion

In summary, our study firstly showed PANDAR was dramatically up-regulated in gastric cancer tissues compared with adjacent normal tissues, and the increased expression of PANDAR was positively correlated with tumor invasion, TNM stage, lymphatic metastasis and patients' outcome.

Conflicts of interest

None.

Acknowledgments

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