Int J Clin Exp Pathol 2015;8(5):5556-5562
www.ijcep.com /ISSN:1936-2625/IJCEP0006544

Original Article

Downregulation of long noncoding RNA ZMAT1 transcript variant 2 predicts a poor prognosis in patients with gastric cancer

Yuexing Lai, Ping Xu, Qinghua Li, Dabin Ren, Jing Wang, Kai Xu, Wei Gao

Department of Gastroenterology, Songjiang Hospital Affiliated First People's Hospital, Shanghai Jiao Tong University, Shanghai 201600, China

Received February 1, 2015; Accepted March 24, 2015; Epub May 1, 2015; Published May 15, 2015

Abstract: Gastric cancer is the second most common cause of cancer-related death partially because of its aggressive metastasis and the fact that it is often diagnosed at an advanced stage. Recent studies have shown that long noncoding RNAs (lncRNAs) play critical roles in multiple biological processes including oncogenesis. In the present study, we found for the first time that the lncRNA ZMAT1 transcript variant 2 is downregulated in gastric cancer tissues compared with adjacent normal tissues ($P < 0.001$). The expression of ZMAT1 transcript variant 2 was inversely correlated with lymph node metastasis ($P < 0.05$), depth of tumor invasion and tumor node metastasis stage ($P < 0.05$). Univariate and multivariate analyses showed that ZMAT1 transcript variant 2 expression was an independent predictor for overall survival ($P < 0.05$). Our study suggests that ZMAT1 transcript variant 2 is a potential diagnostic factor in patients with gastric cancer.

Keywords: Gastric cancer, ZMAT1 transcript, prognosis

Introduction

Despite a marked decline in gastric cancer (GC) incidence in recent years, GC remains the second most frequent cause of cancer-related death partially because of its aggressive metastasis and the fact that it is often diagnosed at an advanced stage [1, 2]. Approximately one half million new cases are diagnosed in China every year, accounting for 42% of the world total [3]. GC is very heterogeneous and patients usually present with different clinical courses and prognoses even at the same clinical stage [4]. Although some biomarkers have been identified as significant prognostic factors for GC [5, 6], few of them have been confirmed as independent predictive factors. Therefore, identifying accurate predictive biomarkers would be of great clinical value for both prediction and improvement of clinical outcome.

The discovery of numerous non-coding RNA (ncRNA) transcripts in human cells has dramatically altered our understanding of the biology of normal and malignant cells. A large number of small ncRNAs, especially microRNAs, have been characterized as oncogenes or tumor suppressors through post-transcriptional regulation of protein expression [7]. By contrast, the functional identities of most long ncRNAs (lncRNAs), which are defined as ncRNAs longer than 200 nucleotides, have not been fully investigated. Mounting evidence indicates that lncRNAs are associated with a diverse range of functions in cell biology [8-11], and the aberrant expressions of lncRNAs have been linked with many types of cancer [12]. LncRNAs play roles in both oncogenic and tumor-suppressive pathways [13] and regulate gene expression at transcriptional, post-transcriptional and epigenetic levels [11, 14, 15]. Altered expression of lncRNAs may potentially promote oncogenesis by altering some of these functions [10, 16]. Thus, to understand the expression and function of lncRNAs may lead to the identification of novel biomarkers and therapeutic targets for cancer.

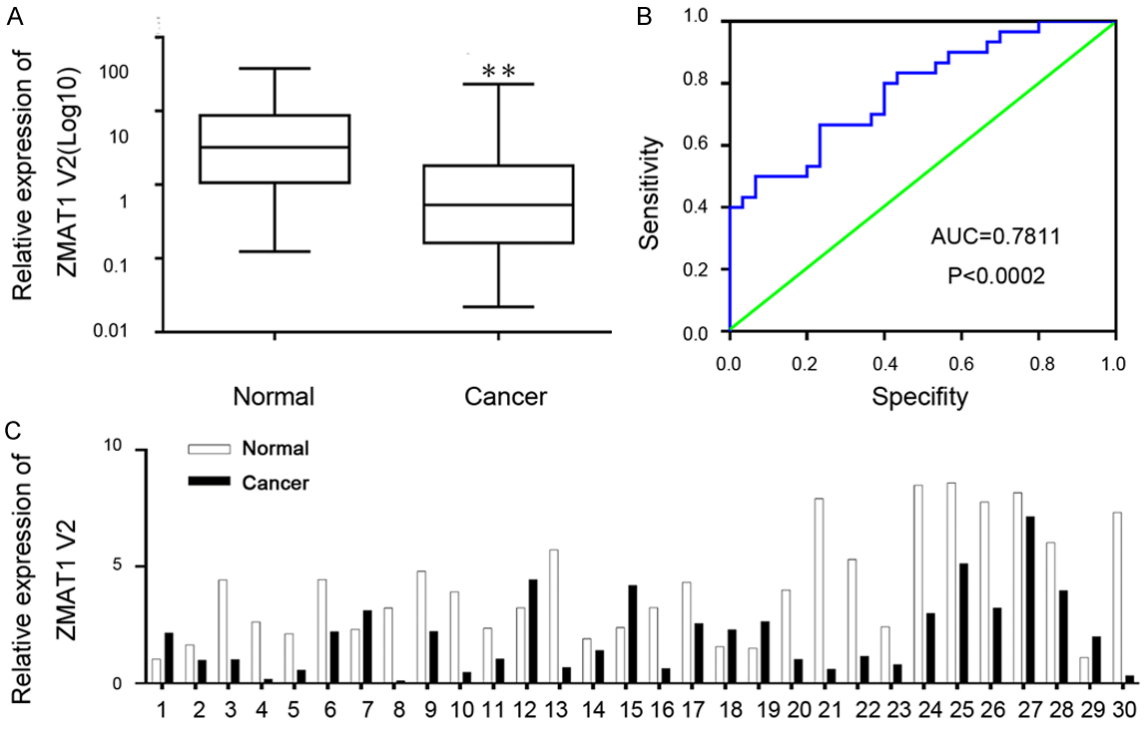

Figure 1. ZMAT1 transcript variant 2 expression in gastric cancer tissues and its clinical significance. A. The relative expression of ZMAT1 transcript variant 2 was quantified by real-time RT-PCR in tumorous and adjacent non-tumorous tissues. B. The relative expression of ZMAT1 transcript variant 2 mRNA in LNs with or without metastasis. C. ROC analysis of ZMAT1 transcript variant 2 expression as a predictor of LN metastasis.

A recent search for lncRNAs that may be involved in the promotion or suppression of the metastatic process identified a number of novel lncRNAs. One of them is zinc finger, matrin-type 1 (ZMAT1) transcript variant 2 (NR_036431). This gene is located in chromosome X: 101,138,612-101,187,000 reverse strand, and has 6 transcripts (splice variants), including ZMAT1-001 (3185 bp), ZMAT1-202 (2367 bp), ZMAT1-201 (3185 bp), ZMAT1-003 (5754 bp), ZMAT1-002 (2654 bp), ZMAT1-003 (755 bp) (www.ensembl.org). The first three transcript variants are protein-coding, whereas the last three, including ZMAT1 transcript variant 2, are non-protein-coding. Whether this gene is associated with cancer remains to be elucidated.

Materials and methods

Patients and samples

A total of 89 patients who underwent D2 radical resection between January 2007 and December 2008 were recruited from our hospital. The study was approved by the Ethics Committee of our hospital. Specimens were obtained immediately after surgical resection and stored at -80°C for further analysis. Lymph nodes (LNs) with or without metastasis were also harvested during gastrectomy. There were 41 men and 48 women, ranging in age from 44 to 78 years, with a median age of 66 years. Tumor stage was defined according to the American Joint Committee on Cancer/International Union Against Cancer tumor, node, metastasis (TNM) classification system (seventh edition). Clinical data such as date of birth, sex, date of surgery, serum CEA level, HP status, tumor size, tumor location and other content of histopathological reports were extracted from a computerized clinical database.

Total RNA extraction, reverse transcription and real-time RT-PCR

Total RNA from tissues and cells was extracted using the Trizol reagent (Invitrogen, CA). Reverse transcription for mRNAs was performed using the M-MLV Reverse Transcriptase (TaKaRa, Dalian, China). The cDNA template was amplified by real-time RT-PCR using the SYBR®

Table 1. Relationship between ZMAT1 transcript variant 2 expression and clinicopathological features

Clinicopathological variable	Number of patients in each group	ZMAT1 transcript variant 2 expression		X^2	*P* value
		Low	High		
All cases	89	69	20		
Age (years)					
< 50	39	27	12	2.743	0.098
> 50	50	42	8		
Gender					
Male	41	33	8	0.382	0.536
Female	48	36	12		
HP					
Positive	48	36	12	0.382	0.536
Negative	41	33	8		
Size of tumor (cm)					
< 5 (small)	35	24	11	2.656	0.103
≥ 5 (large)	54	45	9		
Location of tumor					
Cardia	19	14	5	0.236	0.889
Body	22	17	5		
Antrum	48	38	10		
Depth of tumor invasion					
T1-T2	32	19	13	9.451	0.002
T3-T4	57	50	7		
Lymph node metastasis					
Present	72	61	11	9.140	0.003
Absent	17	8	9		
Liver metastasis					
Absent	59	43	16	2.169	0.141
Present	30	26	4		
Invasion of contiguous organs n (%)					
Yes	37	28	9	0.125	0.724
No	52	41	11		
Vessel invasion					
Negative	45	34	11	0.203	0.652
Positive	44	35	9		
Stage					
I, II	32	19	13	9.451	0.002
III, IV	57	50	7		
Lauren's classification					
Diffuse	22	16	6	0.107	0.743
Intestinal	67	53	14		
Grade of differentiation					
Sell and moderate	35	24	11	2.656	0.103
Poor and not	54	45	9		
Preoperative chemotherapy					
Yes	41	32	9	0.012	0.913
No	48	37	11		
Serum CEA value (μg/L)					
< 5	55	39	16	3.620	0.057
≥ 5	34	30	4		

Premix Dimmer Eraser kit (TaKaRa, Dalian, China). GAPDH was used as an internal control, and ZMAT1 transcript variant 2 values were normalized to GAPDH. Real-time RT-PCR reactions were performed by the ABI7500 system (Applied Biosystems, CA). The relative expression fold change of mRNAs was calculated by the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Comparisons of continuous data were performed by the independent t test or paired t test between the 2 groups, whereas categorical data were analyzed by the chi-square test. Overall survival was analyzed by the Kaplan-Meier method, and the differences between groups were estimated by the log-rank test. Independent prognostic indicators were assessed in the multivariate analysis us-ing Cox's proportional hazard model. All statistical analyses were performed using SPSS for Windows v.16.0 (SPSS, Chicago, IL) and GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA). $P < 0.05$ was considered statistically significant.

Results

ZMAT1 transcript variant 2 is downregulated in gastric cancer tissues

The expression of ZMAT1 transcript variant 2 was assessed in 89 pairs of human gastric cancer and adjacent noncancerous tissues by quantitative real-

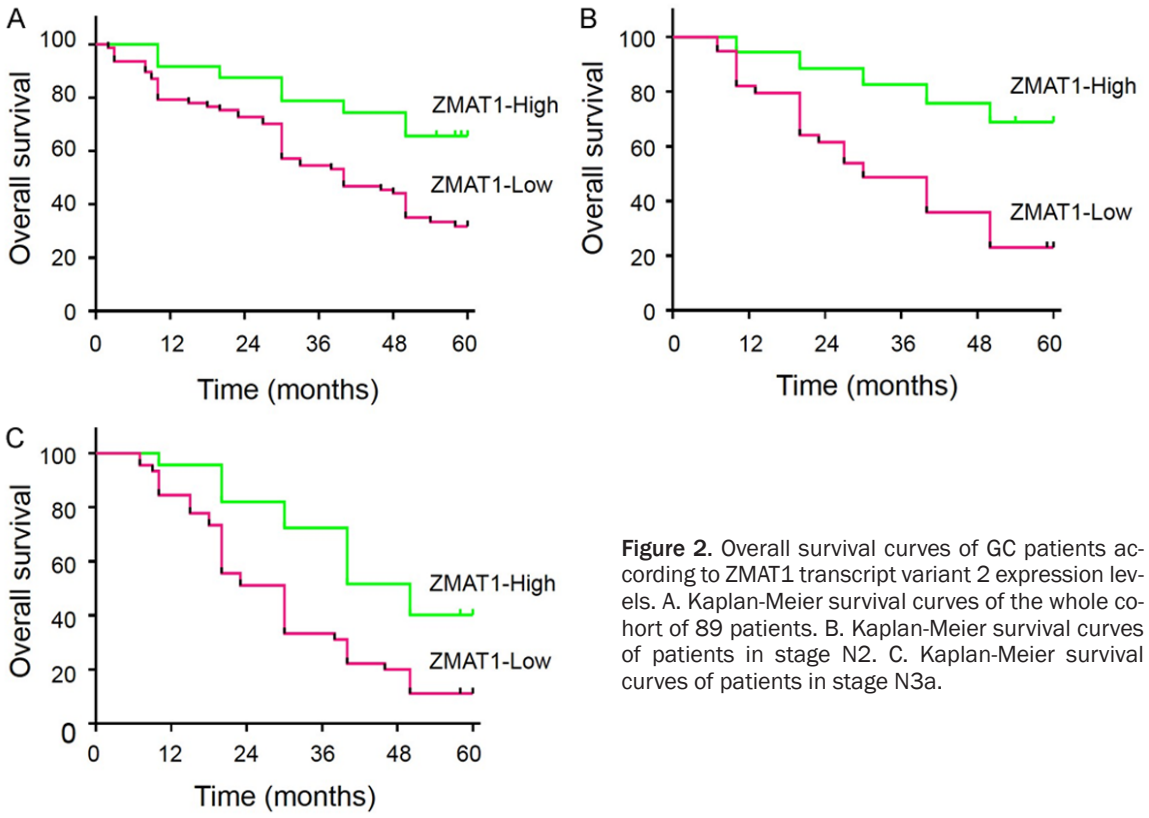

Figure 2. Overall survival curves of GC patients according to ZMAT1 transcript variant 2 expression levels. A. Kaplan-Meier survival curves of the whole cohort of 89 patients. B. Kaplan-Meier survival curves of patients in stage N2. C. Kaplan-Meier survival curves of patients in stage N3a.

time PCR. ZMAT1 transcript variant 2 was downregulated in 69/89 (77.5%) of gastric cancers as compared to their non-tumorous counterparts ($P < 0.005$, **Figure 1A**).

We next evaluated the relationship between ZMAT1 transcript variant 2 expression and various clinicopathological parameters. Our results showed that decreased expression of ZMAT1 was significantly associated with the depth of tumor invasion. The expression level of ZMAT1 transcript variant 2 was inversely correlated with advanced TNM stage ($P < 0.01$). The results of DCt analysis showed that patients with positive lymph node metastasis had low levels of ZMAT1 transcript variant 2 ($P < 0.01$). However, no significant correlation between ZMAT1 transcript variant 2 expression and sex, age, tumor location, tumor size, liver metastasis, Lauren's classification or serum CEA levels was observed (**Table 1**).

ZMAT1 transcript variant 2 downregulation is associated with lymph node metastasis

Lymph node metastasis is one of the most important prognostic factors in patients with GC. To further explore the role of ZMAT1 transcript variant 2 in lymph node metastasis, the expression level of ZMAT1 transcript variant 2 was analyzed in 30 paired LN specimens by real-time RT-PCR. Each paired LN specimen consisted of a lymph node with metastasis and a lymph node without metastasis derived from the same patient. ZMAT1 transcript variant 2 was downregulated in metastatic lymph nodes compared with their matched non-metastatic lymph nodes in 22/30 paired lymph node specimens (70.3%) ($P < 0.05$, **Figure 1B**).

In addition, we explored whether ZMAT1 transcript variant 2 expression status in primary tumors could predict lymph node metastasis. Investigation of predictive values by ROC analysis showed that the area under the curve was 0.781 (**Figure 1C**).

Downregulation of ZMAT1 transcript variant 2 and clinical outcomes

The 1-, 3- and 5-year cumulative survival rates were 92%, 79%, and 66% respectively, for patients with high ZMAT1 transcript variant 2 expression, and 79%, 55% and 32%, respec-

Table 2. Univariate and multivariate analyses of factors associated with overall survival

Clinicopathological variable	Univariate *P*	Multivariate		
		Hazard rate	95% CI	P
Age (years): ≤ 50 versus > 50	0.304			NA
Gender: male versus female	0.543			NA
HP: positive versus negative	0.616			NA
Size: < 5 cm versus ≥ 5 cm	0.497			NA
Location: cardia versus body versus antrum	0.513			NA
Invasion depth: T1-T2 versus T3-T4	0.032	0.944	0.693- 1.286	0.688
LNM: N0 versus N1 versus N2 versus N3a versus N3b	0.004	1.647	1.146-2.367	0.010
Liver metastasis: yes versus no	0.45			NA
ZMAT1: high versus low	0.007	0.68	0.333-1.389	0.03
Invasion of contiguous organs: yes versus no	0.556			NA
Microvessel invasion: yes versus no	0.287			NA
Stage: I, II versus III, IV	< 0.001	0.933	0.570-1.527	0.01
Lauren's classification: diffuse versus intestinal	0.017	1.551	0.804-2.992	0.341
Grade of differentiation: well and moderate versus poor	0.87			NA
Preoperative chemotherapy: yes versus no	0.001	1.678	0.96-2.933	0.16
CEA (μg/mL): ≤ 5 versus > 5	0.067			NA

tively, for those with low ZMAT1 transcript variant 2 expression, indicating that GC patients with low expression of ZMAT1 transcript variant 2 had a poorer prognosis than those with high ZMAT1 transcript variant 2 expression (**Figure 2A**). Univariate analysis indicated that the factors significantly associated with survival were invasion depth, TNM stage, ZMAT1 transcript variant 2 level, preoperative chemotherapy, lymph node metastasis, and Lauren's classification. However, gender, age, and serum CEA levels et al were not related to the prognosis of the patients (**Table 2**). The clinicopathological parameters that were correlated with the survival of the patients in the univariate analysis were included in the multivariate Cox analysis. The results showed that TNM stage, lymph node metastasis, and ZMAT1 transcript variant 2 expression were independent prognostic factors for patients with GC (**Table 2**).

When stratified by lymph node status, most of our patients were at N2 and N3a stages. In both stages, patients with high expression of ZMAT1 transcript variant 2 had significantly longer mean survival times than those with low expression of ZMAT1 transcript variant 2 (**Figure 2B** and **2C**).

Discussion

Mammalian genomes encode thousands of lncRNAs [17], which are generated through pathways similar to those of protein coding genes, indicating that lncRNAs could play important roles in diverse cell signaling pathways. More than 30,000 lncRNAs have been annotated in the FANTOM database and many lncRNAs remain to be identified and characterized. Now a growing line of evidence supports a role for lncRNAs as predictive biomarkers or tumor targets in human cancers [18-21].

In the present study, we found that ZMAT1 transcript variant 2 was significantly decreased in GC and that its expression level was inversely correlated with the aggressive biological behavior of GC. Patients with low ZMAT1 transcript variant 2 expression were at a more advanced stage and more frequently showed lymphovascular infiltration and a shorter disease-free interval than those with high expression. To the best of our knowledge, this is the first study showing the downregulation of ZMAT1 transcript variant 2 expression in gastric cancer.

ZMAT1 transcript variant 2 has not been well characterized and little is known about its expression profile and biological functions. In the present study, we found that decreased ZMAT1 transcript variant 2 expression may be predictive of poor prognosis in gastric cancer; however, the study had some limitations. All the patients enrolled in the study were from the same hospital, and the number of samples was not sufficient for subgroup analysis. These

issues may lead to incorrect conclusions. In a future study, we will increase the number of samples and examine the molecular mechanisms underlying the altered expression of ZMAT1 transcript variant 2 in gastric cancer at a cellular level.

In summary, ZMAT1 transcript variant 2 was downregulated in GC and its expression levels were inversely correlated with lymph node metastasis, distant metastasis, and poor prognosis of patients with GC. These data suggest that ZMAT1 transcript variant 2 is a potential biomarker for the diagnosis of gastric cancer.

Acknowledgements

This work was funded by the medical guide project of Shanghai Municipal Science and Technology Commission (No. 114119b0100), the medical leading project of Songjiang District Health Bureau (No. 2011LX07), the youth scientific research fund of Shanghai Municipal Commission of Health and Family Planning (No. 20144Y0162) and the key medical specialties fund of Shanghai (No. ZK2012A38).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ping Xu, Department of Gastroenterology, Songjiang Hospital Affiliated First People's Hospital, Shanghai Jiao Tong University, Shanghai 201600, China. Tel: +86-2167720376; Fax: +86-21-67720300; E-mail: yfyxp@aliyun.com

References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Hartgrink HH, Jansen EP, van Grieken NC and van de Velde CJ. Gastric cancer. Lancet 2009; 374: 477-490.

[3] Yang L. Incidence and mortality of gastric cancer in China. World J Gastroenterol 2006; 12: 17-20.

[4] Lim L, Michael M, Mann GB and Leong T. Adjuvant therapy in gastric cancer. J Clin Oncol 2005; 23: 6220-6232.

[5] He H, Chen W, Wang X, Wang C, Liu F, Shen Z, Xu J, Gu J and Sun Y. Snail is an independent prognostic predictor for progression and patient survival of gastric cancer. Cancer Sci 2012; 103: 1296-1303.

[6] Yasui W, Oue N, Aung PP, Matsumura S, Shutoh M and Nakayama H. Molecular-pathological prognostic factors of gastric cancer: a review. Gastric Cancer 2005; 8: 86-94.

[7] Farazi TA, Spitzer JI, Morozov P and Tuschl T. miRNAs in human cancer. J Pathol 2011; 223: 102-115.

[8] Kaikkonen MU, Lam MT and Glass CK. Non-coding RNAs as regulators of gene expression and epigenetics. Cardiovasc Res 2011; 90: 430-440.

[9] Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, Thomas K, Presser A, Bernstein BE, van Oudenaarden A, Regev A, Lander ES and Rinn JL. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci U S A 2009; 106: 11667-11672.

[10] Mercer TR, Dinger ME, Sunkin SM, Mehler MF and Mattick JS. Specific expression of long noncoding RNAs in the mouse brain. Proc Natl Acad Sci U S A 2008; 105: 716-721.

[11] Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S and Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010; 464: 1071-1076.

[12] Gutschner T and Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. RNA Biol 2012; 9: 703-719.

[13] Zhou Y, Zhong Y, Wang Y, Zhang X, Batista DL, Gejman R, Ansell PJ, Zhao J, Weng C and Klibanski A. Activation of p53 by MEG3 non-coding RNA. J Biol Chem 2007; 282: 24731-24742.

[14] Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E and Chang HY. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 2007; 129: 1311-1323.

[15] Cheetham SW, Gruhl F, Mattick JS and Dinger ME. Long noncoding RNAs and the genetics of cancer. Br J Cancer 2013; 108: 2419-2425.

[16] Huarte M and Rinn JL. Large non-coding RNAs: missing links in cancer? Hum Mol Genet 2010; 19: R152-161.

[17] Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, Cabili MN, Jaenisch R, Mikkelsen TS, Jacks T, Hacohen N, Bernstein BE, Kellis M, Regev A, Rinn JL and Lander ES. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 2009; 458: 223-227.

[18] Nie L, Wu HJ, Hsu JM, Chang SS, Labaff AM, Li CW, Wang Y, Hsu JL and Hung MC. Long non-coding RNAs: versatile master regulators of gene expression and crucial players in cancer. Am J Transl Res 2012; 4: 127-150.

[19] Prensner JR, Rubin MA, Wei JT and Chinnaiyan AM. Beyond PSA: the next generation of prostate cancer biomarkers. Sci Transl Med 2012; 4: 127rv123.

[20] Salagierski M and Schalken JA. Molecular diagnosis of prostate cancer: PCA3 and TMPRSS2:ERG gene fusion. J Urol 2012; 187: 795-801.

[21] Yang Z, Zhou L, Wu LM, Lai MC, Xie HY, Zhang F and Zheng SS. Overexpression of long non-coding RNA HOTAIR predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation. Ann Surg Oncol 2011; 18: 1243-1250.