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*Original Article*

# Aberrant NEAT1 promotes migration in endometrial cancer and as marker of poor prognosis

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**Abstract:** NEAT1 shares homology with NEAT2 (also known as MALAT-1), which is a well characterized lncRNA that promotes the migration of malignant cells. However, the function of NEAT1 in endometrial cancer (EC) remains unknown. Here, we investigated the NEAT1 expression levels in EC tissue using ISH. The median score of NEAT1 in EC tissues was used as a cutoff value to divided the patient cohort into 2 groups, the high expression and low expression groups for further analysis of the correlation between the NEAT1 levels and the clinical characteristics of EC. Moreover, we silenced NEAT1 in EC cells, and measured cell mobility using the wound healing and transwell assays. We found that the level of NEAT1 was a marker of poor prognosis of EC; elevated NEAT1 levels corresponded to a lower OS and were closely related with metastasis. Knocking down NEAT1 in EC cells significantly inhibited cell mobility. Consequently, our findings suggest that NEAT1 promotes migration and may serve as a predictive marker for EC patients.

**Keywords:** Endometrial cancer, metastasis, long non-coding RNA, NEAT1

## Introduction

Use of the ThinPrep cytological test has decreased the morbidity of cervical cancer. However, endometrial cancer (EC) is the most common female reproductive tract malignancy in both developed and developing countries [1, 2]. EC originates from the endometrium and initially presents with painless vaginal bleeding with the muscular layer serving as a natural barrier. Therefore, women with low- and intermediate-risk EC generally have an excellent prognosis. Nonetheless, up to 30% of women with EC have high-risk tumors that not only spread deep into the myometrium but also metastasize via the lymphatic system, blood vessels, and fallopian tubes [3]. Nearly one in five patients develop unexpected relapses and metastasis to either the pelvic and para-aortic lymph nodes or distant sites such as bone and lung [4]. Individualized medicine has been advocated in oncotherapy, and how to stratify patients with optimal surgical staging based on lymph node metastasis for more reasonable treatment options and better survival outcomes has been successfully implemented [5]. Ultrasound and endometrial sampling are often the first tests performed in diagnosis of EC. However, regarding minimally invasive blood biomarkers, CA125 is the only classic clinical monitoring index for EC to date [6-8]. Recently, HE4 has been reported as a second clinical tumor marker [9, 10]. Another study found that circulating tumor cells may be a potential indicator for recurrence and metastasis [11]. The use of circulating tumor DNA as the dynamic marker has also attracted the attention of researchers [12]. Non-coding RNA comprises almost 98 percent of the whole human genome. Among non-coding RNA, molecules greater than or equal to 200 nt are known as long non-coding RNAs (lncRNAs), which are known regulators of post-translational processing of mRNAs, including splicing, editing, trafficking, translation, degradation, and are involved in cell survival, apoptosis, and metabolism [13-15]. Since over-ex-

pressed lncRNAs are stable in serum and paraffin-embedded tissue, its function as a tumor biomarker has been verified in various malignancies. Nuclear paraspeckle assembly transcript (NEAT) is a nuclear-restricted long noncoding RNA located on chromosome 11 that encodes the two isoforms NEAT1 and NEAT2 and is also known as MALAT1. As an oncogene, MALAT1 has been widely recognized in many cancers, including EC [16, 17]. However, the function of NEAT1 in EC remains poorly studied. Here, we explore the expression levels and function of NEAT1 in primary EC tissues and EC cell lines.

## Materials and methods

### *Patients and tissue samples*

This study was approved by the Research Ethics Committee of Nanfang Hospital and the First Affiliated Hospital of Jinan University. Written informed consent was obtained from all of the patients. Paraffin sections were obtained from 58 patients who were diagnosed with EC and admitted to the Gynecology and Obstetrics Department of Nanfang Hospital and the First Affiliated Hospital of Jinan University between 2007 and 2010. The EC diagnosis was confirmed based on FIGO guidelines and the 2008 WHO classification criteria. All of these patients underwent molecular and phenotypic classification and were distinguished as Type I (mainly endometrioid) or Type II (non-endometrioid). The follow-up, at 60 months, was conducted by mail or phone.

### *In situ hybridization*

ISH was performed on EC paraffin sections using an ISH optimization kit for FFPE (Exiqon, Denmark) in accordance with the manufacturer's instructions. Briefly, after the samples were dewaxed and dehydrated, they were digested by proteinase K and then pre-hybridized for 1 h. A NEAT1 probe (designed with Exiqon Web 5'-AACGCACAAGAAGGCAGGCAA-3') was incubated on the slide at 59°C for 16 h. An anti-digoxigenin antibody (Roche) was preadsorbed at 1:1000 dilution in blocking solution and then applied to the sections for 16 hrs at 4°C, and the slides were subjected to an NBT/BCIP developing solution in the dark at RT for 4 h. Finally, the slides were counterstained with Nuclear Fast Red to visualize the nuclei and then mounted in aqueous mounting medium (Maixin Biotechnology Company, China). The sections were scored manually semiquantitatively for cytoplasmic staining. The staining intensity of the tumor cells was scored as follows: 0 = negative; 1 = weak (0~25%); 2 = intermediate (26~50%); 3 = strong (51~75%); 4 = extra strong (76~100%). We scored five random microscope fields and added the scores together.

### *Cell culture*

HTB-111 and Ishikawa cells were maintained in DMEM (Gibco, Carlsbad, CA, USA) supplemented 10% heat-inactivated fetal bovine serum albumin (Logan, USA) and incubated under conditions of 37°C, 95% humidity, and 5% $CO_2$.

### *shRNA plasmid constructed and transduction*

The chemical synthesis of siRNAs targeting human NEAT1 was conducted by Jima Com with the sequences 5'-GATCCCTAAGCTGTAGAACAT-3'. First, HTB-111 and Ishikawa cells were serum-starved in DMEM without fetal bovine serum for 24 h and the cell lines were then transfected with siRNA-NEAT1 using Lipofectamine 3000. After 6 hours, we replaced the medium with DMEM supplemented with 10% FBS.

### *Wound-healing assay*

To evaluate the effect of NEAT1 in EC cells, the wound healing/scratch assay was performed. HTB-111 and Ishikawa cells were seeded in 6-well plates overnight and then transfected with either siRNA-NEAT1 or NC control for 6 h. After replacing the medium with fresh complete DMEM overnight, the monolayer was scraped with a 200 μL sterile pipette tip. The cells were then washed with PBS twice and cultured with fresh DMEM supplemented with 10% FBS in the presence or absence of the $IC_{50}$ concentration of selumetinib. At 48 h after scratching, photo images of the plates were obtained.

### *Migration assay*

After cells were transfected with siRNA-NEAT1 for 6 h, they were trypsinized with 0.25% trypsin and seeded into the upper layer of a Boyden chamber (Haimen, Jiangsu province, China). After incubating for 8 h, the non-migra-

**Table 1.** Relationship between UCA1 expression and clinicopathological characteristics in patients with endometrial cancer (n = 58)

| Patients character | | Low UCA1 group | High UCA1 group | *P* value |
|---|---|---|---|---|
| Age | < 55 | 18 | 10 | 0.925 |
| | ≥ 55 | 15 | 9 | |
| Lymph node metastasis | Negative | 28 | 8 | 0.029 |
| | Positive | 11 | 11 | |
| Distant metastasis | Negative | 32 | 10 | 0.018 |
| | Positive | 7 | 9 | |
| Vessel invasion | Negative | 27 | 8 | 0.048 |
| | Positive | 12 | 11 | |
| ER | Negative | 8 | 7 | 0.189 |
| | Positive | 31 | 12 | |
| PR | Negative | 19 | 12 | 0.309 |
| | Positive | 20 | 7 | |
| Pathology | Adenocarcinoma | 35 | 17 | 0.552 |
| | Non-adenocarcinoma | 4 | 2 | |
| Stage | I | 14 | 1 | 0.016 |
| | II | 21 | 14 | |
| | III/IV | 4 | 4 | |
| Histological grade | G1 | 15 | 4 | 0.032 |
| | G2 | 23 | 11 | |
| | G3 | 1 | 4 | |

tory cells were removed from the top of the well, and the remaining cells were fixed with 4% paraformaldehyde and stained with crystal violet. Photo images of the plates were obtained.

*Statistical analysis*

The data are expressed as the mean ± SD. One-way ANOVA was used to identify the impact of NEAT1 expression on the clinicopathological factors of EC patients. Patient survival rates were calculated using the Kaplan-Meier method, and statistically significant differences were determined using the log-rank test. All *P* values were two-tailed. *P* values < 0.05 were considered significant. All analyses were performed using SPSS software version 19.0.

## Results

*Patient demographics*

A total of 58 female EC patients were enrolled in this study. Among them, 30 (50.84%) were < 60 years old, and 49 (84.48%) had stage I-II disease. The detailed clinicopathological characteristics of the recruited patients are summarized in **Table 1**. The patients' ages ranged from 29 to 72 with an average age of 60.7 years. After a six-month follow-up, none of the physical examinations indicated the presence of a tumor.

*Over-expression of NEAT1 in EC tissue*

NEAT1 is over-expressed in EC tissues. As shown in **Figure 1**, in the EC tissues, there were either light or dark blue particles in the cell. The images in **Figure 1A** and **1C** were positive, and those in b and d were strongly positive. Both a and b were examined under a 20× binocular microscope, c and d were obtained under a 40× binocular microscope.

*Correlation between NEAT1 expression and clinicopathological characteristics*

We correlated NEAT1 expression levels with the clinicopathological characteristics of patients with EC (**Table 1**). Using the median score of NEAT1 expression (9.57), the 58 EC patients were divided into low and high expression groups (**Figure 2A**). NEAT1 levels were positively correlated to stage (P = 0.016), grade (P = 0.032), distant metastasis (P = 0.029), lymph node metastasis (P = 0.029) and vessel invasion (P = 0.048). However, NEAT1 expression was not correlated to other characteristics such as patient age, ER, PR, and pathological type.

*Correlation between elevated NEAT1 expression levels and poor prognosis in patients with EC*

We also evaluated whether upregulation of NEAT1 was linked to the prognosis of EC patients using Kaplan-Meier analysis. The follow-up period for the studied patients was 60 months. Among the 58 patients, 7 of 39 (82.1%)

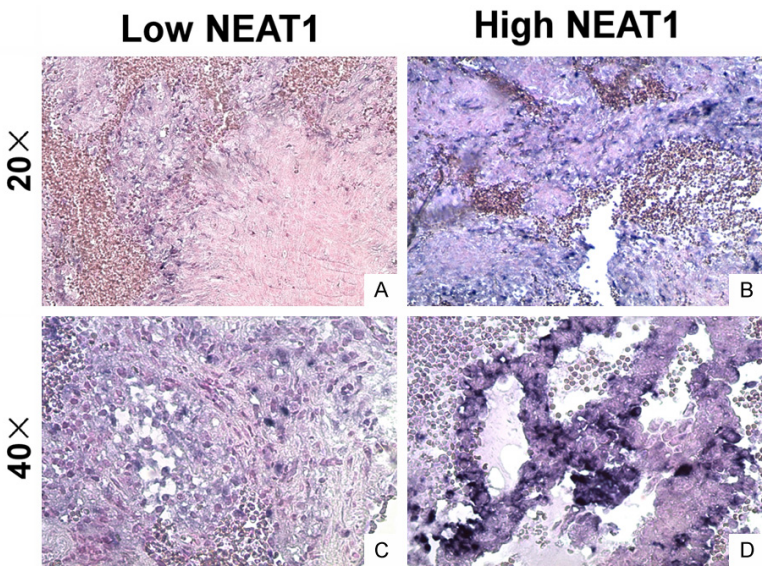


**Figure 1.** Detection of NEAT1 lncRNA expression in EC tissues by ISH. NEAT1 expression in EC tissues is presented as representative images of positive and strongly positive NEAT1 staining using the ISH method. A and C. Show positive NEAT1 staining in EC tissue as light blue fine grains in the cell; B and D. Show strongly positive NEAT1 staining in EC tissue as navy coarse particles in the cell. A and B are at 200× magnification; C and D are at 400× magnification.

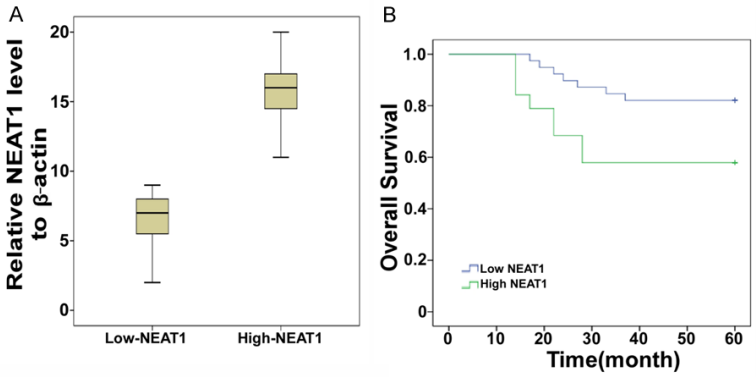


**Figure 2.** NEAT1 is associated with poor prognosis of EC. A. According to the NEAT1 ISH score, the 58 patients were divided into two groups. The median scores of the low and high NEAT1 groups were 6.51 and 15.84, respectively. B. Kaplan-Meier survival analysis of the 58 EC patients indicated that patients with elevated NEAT1 expression had a lower OS (P = 0.032).

with low NEAT1 expression levels died. However, 8 of 19 (52.9%) patients with high NEAT1 expression levels died (P = 0.032; **Figure 2B**).

*NEAT1 regulated the mobility of EC cell*

We found that NEAT1 was over-expressed in EC tissues and was closely related to metastasis. To further investigate the function of NEAT1 on EC, we silenced NEAT1 expression in HTB-111 and Ishikawa cells using siRNA, and the qRT-PCR results verified that the siRNA transfection significantly reduced NEAT1 expression (*indicates P < 0.001, **Figure 3A**). The wound healing assay showed that the siRNA group had a markedly wider wound than the control group. The transwell assay showed that compared with the NC control group, there were dramatically fewer cells treated with siRNA targeting NEAT1 that transmigrated across the membrane.

## Discussion

In recent years, long noncoding RNAs (lncRNAs) have become increasingly studied for their transcriptional regulation of genes. lncRNAs are crucial regulators of various biological processes such as cell cycle, apoptosis, chromatin remodeling, and tumor progression. NEAT1/MENε/β/VINC and MALAT1/NEAT2 are homologous transcription products that localize to nuclear speckles (aka SC35 domains) [18]. However, they localize to different regions: the former localizes to the periphery of SC35, and the latter is found in the interior of all mature SC35 domains. The SC35 domain consists of a specific group of proteins and nucleic acids that are essentially ubiquitous structures and spatially link the expression of specific pre-mRNAs to the rapid recycling of copious RNA metabolic complexes [19]. NEAT functions as a regulator of gene expression by retaining and editing mRNAs in the nucleus [20, 21]. Similar to MALAT1, NEAT1 has also been found to be over-expressed in multiple tumors. Sun [22] reported that NEAT1 is a biomarker for poor prognosis of NSCLC and acts as a competing endogenous RNA (ceRNA) of miR-377-3p due to its three con-

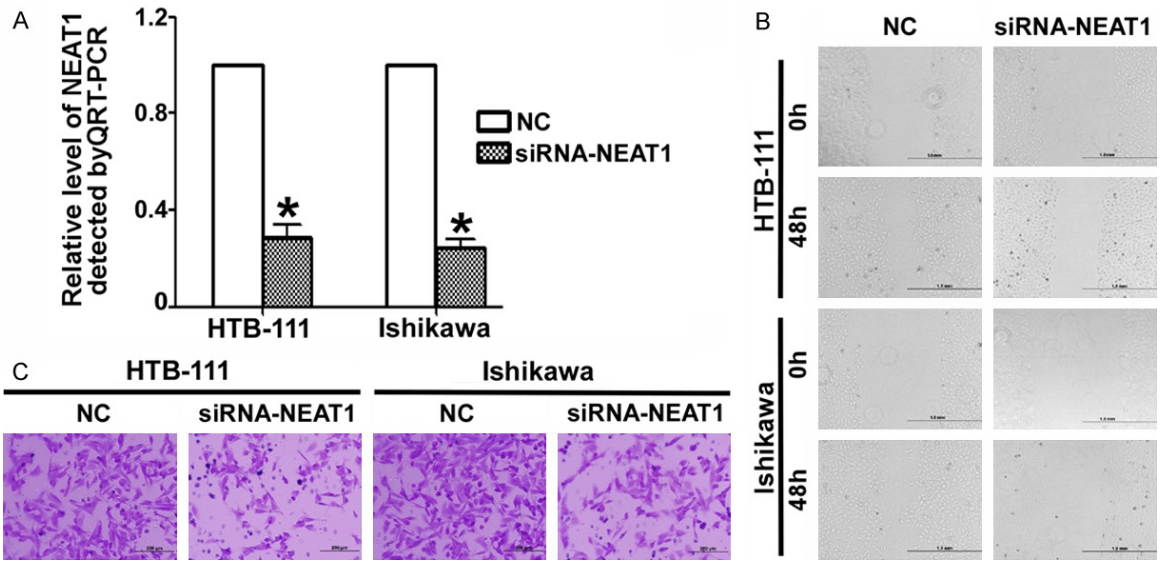


**Figure 3.** NEAT1 regulated the mobility of EC cell lines. A. The NEAT1 levels in HTB-111 and Ishikawa cells were dramatically reduced in cells transfected with siRNA-NEAT1 for 24 h (*P < 0.001 vs NC control); B. The wound healing assay results show that the wound in the siRNA groups was significantly wider than that in the NC control group. C. The assessment of cell mobility via transwell assays showed that the HTB-111 and Ishikawa cells transfected with shRNA-NEAT1 had significantly less migration than the NC controls (*P < 0.001 vs NC control).

served cognate miR-377-3p binding sites. NEAT1 also functions as an oncogene in breast cancer and positively correlates with poor survival in BC. Lo described that BRCA1, which is deficient in BC, binds to the promoter of NEAT1 and inhibits its expression. Meanwhile, NEAT1 down-regulated miR-129-5p by methylating the CpG islands of the miRNA gene, which triggered the activation of oncogenic Wnt signaling. Dysregulation of the BRCA1/NEAT1/miR-129-5p/WNT4 signaling axis contributes to the tumorigenesis of breast cancer [23]. Gernapudi et al [24] found that miR-140 promotes the expression of NEAT1 as well. The miR-140/NEAT1 signaling network is necessary for adipogenesis. NEAT1 also plays an important role in gastric cancer [25] and ovarian cancer tumorigenesis. Here, we first reported NEAT1 expression in EC tissues. The Kaplan-Meier survival assay revealed that elevated NEAT1 expression was closely associated with poor prognosis in EC patients. NEAT1 levels were also positively correlated to stage, grade, distant metastasis, lymph node metastasis and vessel invasion but not ER, PR and pathological type. The analysis of these clinical data indicated the potential role of NEAT1 abnormalities in EC metastasis. Therefore, we knocked down NEAT1 in EC cell lines and detected the change in mobility of HTB-111 and Ishikawa cells. The wound healing and transwell assays suggested that treatment with siRNA-NEAT1 significantly suppressed the migratory ability of EC cells in vitro.

Taken together, our study demonstrated that NEAT1 over-expression was found in EC tissue and plays a carcinogenic role in EC.

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## Disclosure of conflict of interest

None.

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## References


[1] Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: more than two types. Lancet Oncol 2014; 15: e268-278.

[2] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-386.

[3] Li M, Li M, Zhao L, Wang Z, Wang Y, Shen D, Wang J, Wei L. Prior tubal ligation might influence metastatic spread of nonendometrioid endometrial carcinoma. Int J Gynecol Cancer 2016; 26: 1092-1097.

[4] Wu QJ, Li YY, Tu C, Zhu J, Qian KQ, Feng TB, Li C, Wu L, Ma XX. Parity and endometrial cancer risk: a meta-analysis of epidemiological studies. Sci Rep 2015; 5: 14243.

[5] Bendifallah S, Daraï E, Ballester M. Predictive modeling: a new paradigm for managing endometrial cancer. Ann Surg Oncol 2016; 23: 975-988.

[6] Chen Y, Ren YL, Li N, Yi XF, Wang HY. Serum human epididymis protein 4 vs carbohydrate antigen 125 and their combination forendometrial cancer diagnosis: a meta-analysis. Eur Rev Med Pharmacol Sci 2016; 20: 1974-1985.

[7] Nisenblat V, Bossuyt PM, Shaikh R, Farquhar C, Jordan V, Scheffers CS, Mol BW, Johnson N, Hull ML. Blood biomarkers for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev 2016; CD012179.

[8] Lee J, Kong TW, Paek J, Chang SJ, Ryu HS. Predicting model of lymph node metastasis using preoperative tumor grade, transvaginal ultrasound, and serum CA-125 level in patients with endometrial cancer. Int J Gyecol Cancer 2016; 26: 1630-1635.

[9] Dilley J, Gentry-Maharaj A, Menon U. Gynecological surveillance in high risk women. Minerva Ginecol 2016; 68: 497-508.

[10] Brennan DJ, Hackethal A, Mann KP, Mutz-Dehbalaie I, Fiegl H, Marth C, Obermair A. Serum HE4 detects recurrent endometrial cancer in patients undergoing routine clinical surveillance. BMC Cancer 2015; 15: 33.

[11] Ni T, Sun X, Shan B, Wang J, Liu Y, Gu SL, Wang YD. Detection of circulating tumour cells may add value in endometrial cancer management. Eur J Obstet Gynecol Reprod Biol 2016; 207: 1-4.

[12] Pereira E, Camacho-Vanegas O, Anand S, Sebra R, Catalina Camacho S, Garnar-Wortzel L, Nair N, Moshier E, Wooten M, Uzilov A, Chen R, Prasad-Hayes M, Zakashansky K, Beddoe AM, Schadt E, Dottino P, Martignetti JA. Personalized circulating tumor dna biomarkers dynamically predict treatment response and survival in gynecologic cancers. PLoS One 2015; 10: e0145754.

[13] Lakhotia SC. Long non-coding RNAs coordinate cellular responses to stress. Wiley Interdiscip Rev RNA 2012; 3: 779-796.

[14] Alvarez ML, Distefano JK. The role of non-coding RNAs in diabetic nephropathy: potential applications as biomarkers for disease development and progression. Diabetes Res Clin Pract 2013; 99: 1-11.

[15] Lavorgna G, Vago R, Sarmini M, Montorsi F, Salonia A, Bellone M. Long non-coding RNAs as novel therapeutic targets in cancer. Pharmacol Res 2016; 110: 131-138.

[16] Zhao Y, Yang Y, Trovik J, Sun K, Zhou L, Jiang P, Lau TS, Hoivik EA, Salvesen HB, Sun H, Wang H. A novel wnt regulatory axis in endometrioid endometrial cancer. Cancer Res 2014; 74: 5103-5117.

[17] Li Q, Zhang C, Chen R, Xiong H, Qiu F, Liu S, Zhang M, Wang F, Wang Y, Zhou X, Xiao G, Wang X, Jiang Q. Disrupting MALAT1/miR-200c sponge decreases invasion and migration in endometrioid endometrial carcinoma. Cancer Lett 2016; 383: 28-40.

[18] Smolle MA, Bullock MD, Ling H, Pichler M, Haybaeck J. Long non-coding RNAs in endometrial carcinoma. Int J Mol Sci 2015; 16: 26463-26472.

[19] Hutchinson JN, Ensminger AW, Clemson CM, Lynch CR, Lawrence JB, Chess A. A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains. BMC Genomics 2007; 8: 39.

[20] Hall LL, Smith KP, Byron M, Lawrence JB. Molecular anatomy of a speckle. Anat Rec A Discov Mol Cell Evol Biol 2006; 288: 664-675.

[21] He C, Jiang B, Ma J, Li Q. Aberrant NEAT1 expression is associated with clinical outcome in high grade glioma patients. APMIS 2016; 124: 69-74.

[22] Sun C, Li S, Zhang F, Xi Y, Wang L, Bi Y, Li D. Long non-coding RNA NEAT1 promotes non-small cell lung cancer progression through regulation of miR-377-3p-E2F3 pathway. Oncotarget 2016; 7: 51784-51814.

[23] Lo PK, Zhang Y, Wolfson B, Gernapudi R, Yao Y, Duru N, Zhou Q. Dysregulation of the BRCA1/long non-coding RNA NEAT1 signaling axis contributes to breast tumorigenesis. Oncotarget 2016; 7: 65067-65089.

[24] Gernapudi R, Wolfson B, Zhang Y, Yao Y, Yang P, Asahara H, Zhou Q. MicroRNA 140 promotes expression of long noncoding RNA NEAT1 in adipogenesis. Mol Cell Biol 2015; 36: 30-38.

[25] 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.