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 IncRNA 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 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 IncRNAs 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 non-coding 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’-AAGCGACAGAGCGAGCGCAA-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% CO2.

shRNA plasmid constructed and transduction

The chemical synthesis of siRNAs targeting human NEAT1 was conducted by Jima Com with the sequences 5’-GATCCCTAAGCTTAGACAT-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 IC50 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-
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%)...
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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 non-coding 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-
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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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