Role of long non-coding RNA NEAT1 in the prognosis of prostate cancer patients

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Abstract
Prostate cancer is the second leading cause of cancer-related deaths among male population worldwide, its incidence and lethality steadily increase. Nuclear enriched abundant transcript 1 (NEAT1) is a long non-coding RNA (lncRNA), located on chromatin 11. It has been found to function as an oncogene in different kinds of cancer. However, until now, the clinical significance of NEAT1 has not been investigated in prostate cancer.

Paired tissue specimens of prostate cancer and matched normal prostate tissues were obtained from 130 patients with prostate cancer between 2014 and 2019 at The Fourth Affiliated Hospital Zhejiang University, School of Medicine. Group means were compared using the Student t test. Chi-Squared test was used for analyzing the correlation of the expression of NEAT1 with clinicopathologic features of prostate cancer patients. Survival data was analyzed using the Kaplan–Meier estimate and log-rank P was calculated. Cox regression model was used for univariate and multivariate analysis for factors related to overall survival.

The expression of NEAT1 was increased significantly in prostate cancer tissues, compared with adjacent normal prostate tissues (P < .001). NEAT1 expression was significantly associated with TNM stage (P = .005), lymph nodes metastasis (P = .005), distant metastasis (P = .003), and Gleason score (P = .001). Overall survival rate was significantly lower for prostate cancer patients with a high expression level of NEAT1 than with a low NEAT1 expression level (P = .048). In multivariate analysis, the results showed that the expression of NEAT1 was an independent prognostic factor for overall patient survival (HR: 2.111, CI: 1.735–10.295, P = .039).

In the present study, NEAT1 is identified as an important lncRNA that may predict the prognosis of patients with prostate cancer.

Abbreviations: 3'-UTR = 3'-untranslated region, CRC = colorectal cancer, HCC = hepatocellular carcinoma, IncRNA = long non-coding RNA, NEAT1 = nuclear enriched abundant transcript 1, NSCLC = non-small-cell lung carcinoma, RCC = renal cell carcinoma.

Keywords: expression, IncRNA, long non-coding RNA, NEAT1, nuclear enriched abundant transcript 1, prognosis, prostate cancer

1. Introduction
Prostate cancer is the second leading cause of cancer-related deaths among male population worldwide, its incidence and lethality steadily increase. In early-stage prostate cancer, patients may be cured using radical prostatectomy, whereas it remains a challenge to cure patients with metastatic disease. In the early stages, metastatic prostate cancer is sensitive to androgen ablation therapy; however, the majority of cases progress to an androgen-independent stage, which is a primary cause for prostate cancer-associated mortality. Therefore, it is imperative to identify novel potential biomarkers for the prognosis and prevention of the induction and progression of prostate cancer.

Long noncoding RNA (lncRNA) are non-coding RNAs of length above 200 nucleotides that can directly target and regulate DNA, RNA, and protein targets, by competing with endogenous RNA networks. They can regulate a great variety of biological processes, including cell proliferation, differentiation, migration, apoptosis, development, and metabolism. LncRNAs, that are upregulated in cancer tissues, may function as oncogenes by targeting tumor suppressor genes, whereas lowly expressed lncRNAs may act as tumor suppressors by negatively regulating the expression of oncogenes.

Nuclear enriched abundant transcript 1 (NEAT1) is a long non-coding RNA, located on chromatin 11. NEAT1 drives tumor initiation and progression by modulating the expression of genes involved in the regulation of tumor cell growth, migration, invasion, metastasis, epithelial-to-mesenchymal transition, stem cell-like phenotype, chemoresistance, and radioresistance, indicating the potential for NEAT1 to be a novel diagnostic biomarker and therapeutic target. It has been found to function as an oncogene in different kinds of cancer, including gastric cancer, hepatocellular carcinoma, breast cancer, lung cancer, bladder cancer, endometrial cancer, multiple myeloma, melanoma, and so on. NEAT1 was also found to play an oncogene role in prostate cancer by acting on a number of downstream genes. However, until now, the clinical significance of NEAT1 has not been investigated in prostate cancer.
2. Materials and methods

2.1. Patients and clinical tissue samples

Paired tissue specimens of prostate cancer and matched normal prostate tissues were obtained from 130 patients with prostate cancer between 2014 and 2019 at Department of Urology, The Fourth Affiliated Hospital Zhejiang University, School of Medicine, Yiwu. All the patients received extraperitoneal laparoscopic radical prostatectomy. All the tissues were obtained at the time of surgery and immediately stored in liquid nitrogen until use. Histological confirmation of prostate cancer and normal prostate tissue (from peripheral zone of prostate) by a dedicated uropathologist in 5 μm-thick stained section, fresh-frozen tissue fragments were trimmed to maximize the yield of target cells. These patients did not receive adjuvant treatment including radiotherapy or chemotherapy prior to surgery. Written informed consent was obtained from all the patients. The protocol was approved by the Institutional Research Ethics Committee of The Fourth Affiliated Hospital Zhejiang University, School of Medicine.

2.2. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

Total RNA from tissues were isolated by a TRIzol reagent (Invitrogen; Thermo Scientific, Inc., Waltham, MA, USA) according to the manufacturer’s protocol. The transcribed cDNA was used for PCR amplification and its system was configured according to the manufacturer’s instructions. PCR reaction conditions were: Pre-denaturation at 95°C for 10 minutes, denaturation at 95°C for 30 seconds, annulling at 60°C for 30 seconds and elongation at 74°C for 30 seconds for a total of 40 cycles. With Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal reference, the relative expression of each group was calculated using \( 2^{-\Delta\Delta Cq} \) and 3 reactions were performed for each sample. The primer sequences used in this study were as follows: lncRNA NEAT1, F: 5'-CAGGAGAGCCGGCTGCGTAATC-3', R: 5'-CGTGGACATCTGGCCACGCA-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTG-3', R: 5'-ATCCGTTGACTCCGACCTCAC-3'.

2.3. Statistical analysis

Statistical analyses were performed using SPSS software version 18.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism version 6.07 (GraphPad Software, Inc., La Jolla, CA, USA). Group means were compared using the Student t test. Chi-Squared test was used for analyzing the correlation of the expression of NEAT1 with clinicopathologic features of prostate cancer patients. Survival data was analyzed using the Kaplan–Meier estimate and log-rank \( P \) was calculated. Cox regression model was used for univariate and multivariate analysis for factors related to overall survival. \( P < .05 \) was considered to indicate a statistically significant difference.

3. Results

3.1. The expression level of NEAT1 in human prostate cancer

In order to explore the role of NEAT1 in prostate cancer carcinogenesis, the expression patterns of NEAT1 in 130 pairs of human prostate cancer tissues and adjacent normal prostate tissues were analyzed using qRT-PCR. The results showed that the expression of NEAT1 was significantly increased in prostate cancer tissues, compared with adjacent normal prostate tissues (shown in Fig. 1, \( P < .001 \)). To evaluate the correlation between NEAT1 expression and clinicopathological characteristics, the 130 prostate cancer patients were classified into 2 groups according to the median expression of NEAT1.

3.2. Correlation of NEAT1 expression with clinicopathological features of prostate cancer

We compared the clinicopathological factors of the high NEAT1 expression group (n=64) and low NEAT1 expression group (n=66). As shown in Table 1, high NEAT1 expression was significantly associated with TNM stage (\( P = .005 \)), lymph nodes
metastasis ($P = .005$), distant metastasis ($P = .003$), and Gleason score ($P = .001$). However, no statistically significant correlation was observed between NEAT1 expression and patients age, serum PSA level, and pt-stage (all $P > .05$).

### 3.3. Relationship between the expression level of NEAT1 and prognosis in patients with prostate cancer

The relationship between the expression level of NEAT1 and the overall survival rate after surgery was examined. Overall survival rate was significantly lower for prostate cancer patients with a high expression level of NEAT1 than those with a low NEAT1 expression level ($P = .048$, shown in Fig. 2). In multivariate analysis, the results showed that the expression of NEAT1 was an independent prognostic factor for overall patient survival (HR: 2.111, CI: 1.735–10.295, $P = .039$, shown in Table 2).

### 4. Discussion

Prostate cancer is a common malignancy in male patients.[25,26] Signaling pathways mediated by androgen and its receptors play a key role in the growth and development of the prostate. Currently, the main treatment method for prostate cancer is to inhibit the secretion of androgens in patients by surgery or drugs, thereby reducing androgen level. However, nearly all such patients develop castration-resistant prostate cancer after primary androgen deprivation therapy. Meanwhile, various mutations have been found to be associated with prostate cancer progression.[27] Therefore, it is urgently necessary to investigate the mechanism behind prostate cancer progression because this accomplishment will help to devise effective strategies for the diagnosis, treatment, and prognosis of prostate cancer.[28,29]

NEAT1 is a long non-coding RNA, located on chromatin 11. It has been found to function as an oncogene in different kinds of cancer. For example, Xia et al found that NEAT1 was upregulated in gastric cancer tissues, promoted proliferation, and inhibited apoptosis of gastric cancer cells. NEAT1 could directly bind to and negatively regulate miR-497–5p expression. PIK3R1 was then identified as a downstream target of miR-497–5P. PIK3R1 was found to be directly negatively regulated by miR-497–5p and indirectly positively regulated by NEAT1. Finally, NEAT1 knockdown inhibited tumor growth, increased miR-497–5p expression, and decreased PIK3R1 expression in xenograft model mice compared with the negative control. These results provided valuable insights into the underlying regulation signaling in gastric cancer development, shedding light on NEAT1 a promising therapeutic target from bench to clinic.[17] Li et al found that NEAT1 could be a sponge for...

**Table 2**

| Variable                        | Hazard ratio | 95% CI     | $P$ value |
|---------------------------------|--------------|------------|-----------|
| Age                             | 1.383        | 0.772–3.293| .288      |
| Pt-stage                        | 2.103        | 0.883–4.285| .192      |
| Preoperative PSA                 | 1.293        | 0.831–2.384| .105      |
| TNM stage                       | 2.448        | 1.936–10.927| .006     |
| Lymph node metastasis           | 2.839        | 1.728–11.293| .013     |
| Distant metastasis              | 3.203        | 2.018–8.295| .009      |
| Gleason score                   | 2.034        | 1.036–4.022| .017      |
| NEAT1 expression level          | 2.111        | 1.735–10.295| .039     |
we need to study the specific mechanism of its role in prostate cancer. Secondly, we only investigated it clinical significance in Asian population, further investigation should be performed in other race.

In conclusion, NEAT1 is identified as an important lncRNA that may predict the prognosis of patients with prostate cancer.

**Author contributions**

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