

Long non-coding RNA NORAD induces cell proliferation and migration in prostate cancer

Journal of International Medical Research

2019, Vol. 47(8) 3898–3904

© The Author(s) 2019

Article reuse guidelines:

sagepub.com/journals-permissions

DOI: 10.1177/0300060519862076

journals.sagepub.com/home/imr



Haiyan Zhang and Haixiang Guo 

Abstract

Objectives: Prostate cancer (PCA) is the deadliest urological disease affecting men worldwide. Long noncoding RNA activated by DNA damage (NORAD) levels are increased in many cancer types, and induce cancer cell progression. However, little is known about the biological functions of NORAD in PCA.

Methods: In this work, the roles of NORAD in cell proliferation, migration, and apoptosis were examined by Cell Counting Kit-8, scratch wound, and annexin V-fluorescein isothiocyanate/propidium iodide staining assays, respectively, in PCA cell lines. Knockdown of NORAD was achieved by small interfering (si)RNA in PCA cell lines, and quantitative real-time PCR was used to detect the expression of NORAD.

Results: Cell proliferation and migration rates were significantly lower in the siNORAD group than in the wild-type group, while the apoptosis level was significantly higher in the siNORAD group compared with the wild-type group.

Conclusions: These results suggest that NORAD promotes the proliferation and migration of PCA cells and inhibits their apoptosis.

Keywords

NORAD, prostate cancer, proliferation, migration, long non-coding RNA, apoptosis

Date received: 9 March 2019; accepted: 17 June 2019

Introduction

Prostate cancer (PCA) is the most frequently occurring cancer in men, and the leading mortality risk to men's health.^{1,2}

Department of Urology, The Second Affiliated Hospital of Kunming Medical University, Kunming Yunnan, China

Corresponding author:

Haixiang Guo, Department of Urology, The Second Affiliated Hospital of Kunming Medical University, Kunming Yunnan 650101, China.

Email: haixiangguo1234@163.com



Moreover, the number of newly diagnosed cases of PCA is increasing in most countries.³⁻⁵ Despite the treatment options currently available for patients with early-stage PCA, such as surgery and radiation therapy, the 5-year survival rate is very low for patients with later-stage disease.^{6,7} Therefore, an understanding of the mechanisms of PCA development is urgently needed.

Long noncoding RNAs (lncRNAs) are nonprotein coding RNAs >200 nucleotides in length.⁸⁻¹⁰ Several lncRNAs, including GAS5,¹¹ LRIG3,¹² and DCTPP1,¹³ were previously shown to participate in the progression of PCA. Recently, long noncoding RNA activated by DNA damage (NORAD) was reported to be overexpressed in a range of cancer types such as pancreatic cancer¹⁴ and bladder cancer,¹⁵ with high NORAD levels being associated with a low survival rate.

NORAD induces the invasion and metastasis of pancreatic carcinoma cells, NORAD levels were found to be higher in colorectal cancer (CRC) tissues than in control samples,¹⁶ and NORAD is required for CRC progression through the targeting of miR-202-5p.¹⁷ However, the expression pattern and function of NORAD in PCA remain unknown. Therefore, the present study aimed to analyze the role of NORAD in PCA.

Materials and methods

Materials

The human PCA cell lines LNCaP, 22Rv1, PC-3, and DU145 and normal prostate epithelial cells (RWPE-1) were purchased from the American Type Culture Collection (Manassas, VA, USA). RPMI-1640 medium was purchased from Thermo Fisher Scientific (Waltham, MA, USA) and Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA, USA).

Cell culture

PCA cells were cultured in RPMI-1640 medium with 10% fetal bovine serum (FBS) and 1% penicillin G and streptomycin (Gibco, Grand Island, NY, USA) in an atmosphere of 5% CO₂ at 37°C. This study included no animal or human experiments, so ethical permission was not required.

Cell transfection

NORAD small interfering (si)RNA (si-NORAD) was provided by Heyuan (Shanghai, China). PCA cells (1×10^5) were seeded in 24-well plates. After 24 hours, they were transfected with 10 nM si-NORAD using 1 μ l Lipofectamine 2000 according to the manufacturer's protocol. They were then incubated for a further 48 hours before being harvested for further investigation.

Cell proliferation assay

PCA cell proliferation was assessed using a Cell Counting Kit (CCK)-8 assay (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) according to the manufacturer's instructions. Briefly, PCA cells (5×10^3) were added to each well of 96-well plates for 0 to 5 days, then 10 μ l of CCK-8 reagent was added per well and incubated for 2 hours at 37°C. The absorbance of each well at 450 nm was analyzed by an enzyme immunoassay analyzer.

Annexin V- fluorescein isothiocyanate (FITC)/propidium iodide (PI) staining assay

The level of NORAD was measured 48 hours after transfection, and PC-3 and DU-145 cells were harvested for apoptosis assays. The PCA cell apoptosis rate was measured using an annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis kit (BD Biosciences,

San Diego, CA, USA) in accordance with the manufacturer's instructions. At least 1×10^4 cells were dyed with Annexin V-FITC and PI for 15 minutes, then evaluated using a flow cytometer (BD Biosciences).

Migration assay

Cells (5×10^5) were plated into 6-well plates and grown to 90% confluence. A 35-mm scratch on the surface of the cells was generated using a plastic tip, then cells were cultured for a further 24 hours in new medium containing 0.5% FBS. Finally, scratch images were acquired using a microscope (Olympus, Tokyo, Japan).

Reverse transcription PCR

Total RNA was isolated using TRIzol reagent (Life Technologies, Shanghai, China). cDNA was synthesized using the Prime Script™ RT Reagent Kit (Takara, Dalian, China), then quantitative real-time (qRT)-PCR was performed with the VeriQuest SYBR Green qPCR Master Mix kit (Thermo Fisher Scientific) using GAPDH as an internal mRNA control. The relative level of NORAD was evaluated using the $2^{-\Delta\Delta C_t}$ method.

Statistical analysis

Data were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The statistical significance of differences between groups was determined using t tests. Data are expressed as means \pm standard deviations. Western blot findings were evaluated using Quantity One software (Bio-Rad, Kunming, China). $P < 0.05$ was considered significant.

Results

Analysis of NORAD expression in PCA cell lines

To study the effect of NORAD on PCA, we first detected NORAD levels by qRT-PCR in PCA cells. NORAD expression was significantly higher in the four human PCA cell lines than in control cells (Figure 1; $P < 0.05$). The highest expression of NORAD was detected in PC-3 and DU-145 cells ($P < 0.01$), so these were used in subsequent experiments.

NORAD knockdown in PCA cells

To further investigate the role of NORAD in PCA, we established a PCA cell model by transfecting siRNA targeting NORAD. We used qRT-PCR to measure mRNA expression. As shown in Figure 2, NORAD mRNA expression was significantly reduced following siRNA transfection ($P < 0.05$), indicating that NORAD was downregulated in PC-3 and DU145 cell lines.

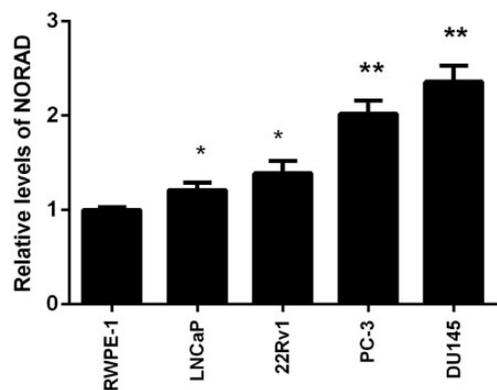


Figure 1. NORAD expression is higher in LNCaP, 22Rv1, PC-3, and DU145 cell lines than in healthy prostate epithelial cells. Data are representative of three experiments; results are means \pm SD. * $P < 0.05$, ** $P < 0.01$.

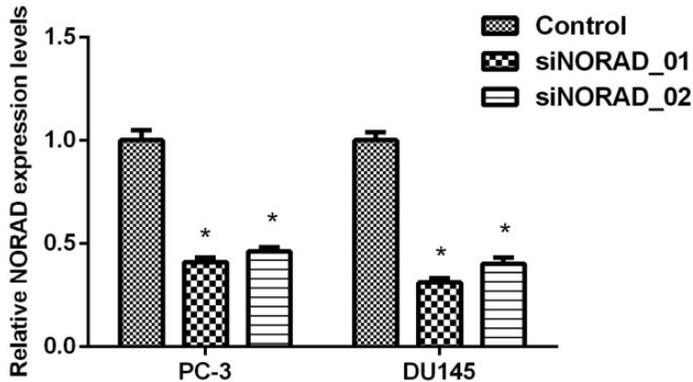


Figure 2. NORAD was knocked down by siRNA in PC-3 and DU145 cells. qRT-PCR detected the inhibitory efficiency of siRNA and si-control. Data are representative of three experiments; results are means \pm SD. * $P < 0.05$.

Effects of NORAD on PC3 and DU145 cell proliferation

Next, we assessed the proliferation of PC-3 and DU145 cells after NORAD silencing. The CCK-8 assay showed that proliferation was significantly lower in the si-NORAD group than in the si-NC group (Figure 3a and b; $P < 0.05$ and 0.01). These results suggested that NORAD is involved in PCA cell proliferation.

Effects of NORAD on PC3 and DU145 cell apoptosis rate

Apoptosis in PC-3 and DU145 cells was also assessed after NORAD silencing. Flow cytometry analysis revealed that NORAD knockdown cells had a significantly increased apoptosis rate compared with si-NC cells (Figure 4; $P < 0.05$). This indicated that NORAD is involved in PCA cell apoptosis.

Effect of NORAD on PC3 and DU145 cell migration

To assess the metastasis of PC-3 and DU145 cells after NORAD silencing, the wound healing assay was used. The PCA cell migration rate was significantly decreased after

NORAD knockdown compared with the si-NC group of cells (Figure 5; $P < 0.05$).

Discussion

Despite many advances in early diagnosis and treatment strategy developments, PCA remains a lethal disease. In this study, our data showed that NORAD expression was induced in PCA cell lines, which consequently increased their proliferation and migration ability. These results imply the existence of a mechanistic relationship between NORAD and PCA.

According to past research, NORAD overexpression is associated with poor prognosis in many types of cancer patients.^{18–20} NORAD is extensively overexpressed in cervical cancer, which induces cervical cancer progression. Additionally, NORAD induces the transforming growth factor- β pathway and modulates the progression of epithelial–mesenchymal transition progression in lung cancer.²¹

To the best of our knowledge, no previous study has investigated the effect of NORAD on PCA cells. To examine the impact of NORAD on PCA, its expression was knocked down in PC3 and DU145 cells using siRNA. Consistent with a previous

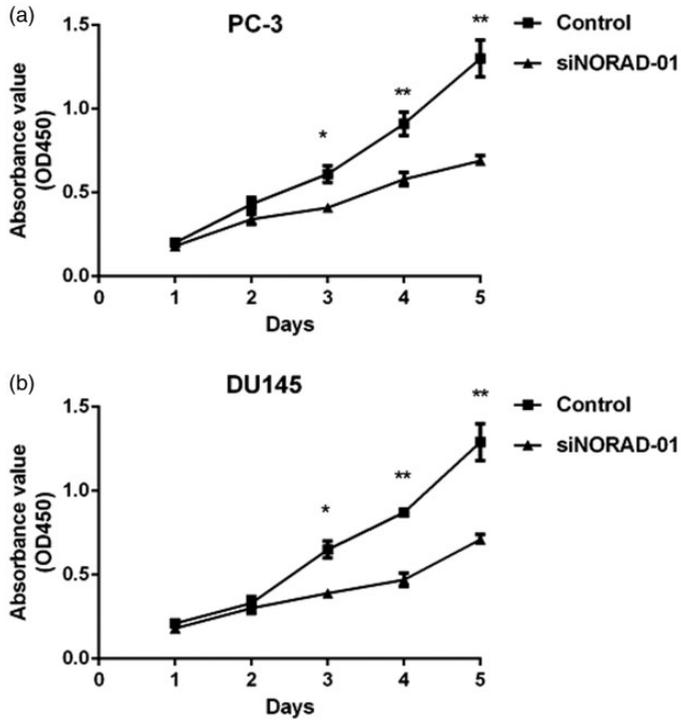


Figure 3. Knockdown of NORAD reduced PC-3 (a) and DU145 (b) cell proliferation according to a CCK-8 assay. Data are representative of three experiments; results are means \pm SD. * $P < 0.05$, ** $P < 0.01$.

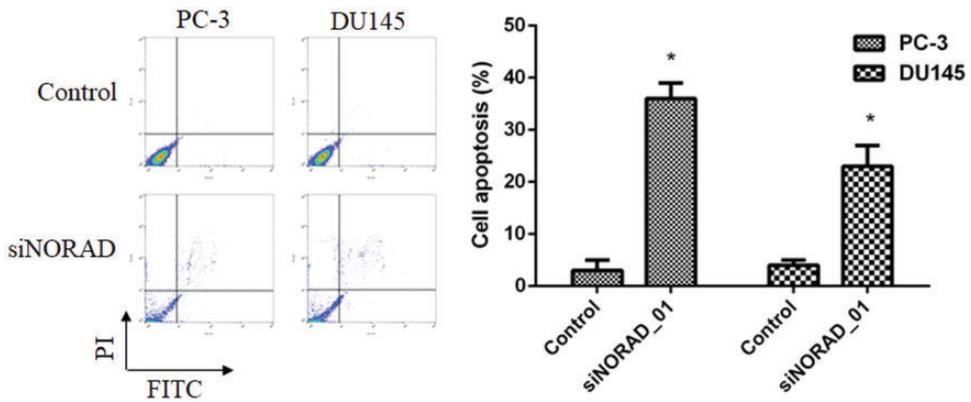


Figure 4. Knockdown of NORAD induced PC-3 and DU145 cell apoptosis according to an annexin V-FITC/PI assay. Data are representative of three experiments; results are means \pm SD. * $P < 0.05$.

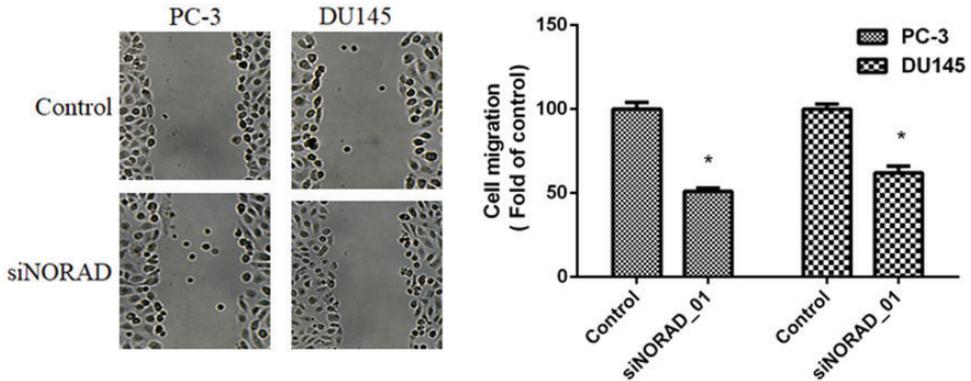


Figure 5. Knockdown of NORAD decreased PC-3 and DU145 cell migration according to a wound healing assay. Data are representative of three experiments; results are means \pm SD.

* $P < 0.05$.

report,²² our observations indicated that silencing NORAD significantly suppressed the PCA cell proliferation and migration capacity and increased cell apoptosis.

In conclusion, we found that NORAD facilitated the metastasis and proliferation of PCA. However, although our data provide new insights into the roles of NORAD in PCA cells, the underlying mechanisms require further investigation.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ORCID iD

Haixiang Guo  <https://orcid.org/0000-0002-0482-984X>

References

1. Fenton JJ, Weyrich MS, Durbin S, et al. Prostate-specific antigen-based screening for prostate cancer: evidence report and systematic review for the US preventive services task force. *JAMA* 2018; 319: 1914–1931.
2. Foerster B, Pozo C, Abufaraj M, et al. Association of smoking status with recurrence, metastasis, and mortality among patients with localized prostate cancer undergoing prostatectomy or radiotherapy: a systematic review and meta-analysis. *JAMA Oncol* 2018; 4: 953–961.
3. Zhao F, Shen J, Yuan Z, et al. Trends in treatment for prostate cancer in china: preliminary patterns of care study in a single institution. *J Cancer* 2018; 9: 1797–1803.
4. Pan Y, Liu B, Huang Y, et al. Characteristics of prostate cancer detection rate (PCDR) in Chinese Han population under different prostate biopsy methods. *Oncotarget* 2017; 8: 32930–32936.
5. Beck V, Schlenker B, Herlemann A, et al. The increase of stage, grading, and metastases in patients undergoing radical prostatectomy during the last decade. *World J Urol* 2019; 37: 1103–1109.
6. Crehange G, Izaguirre A, Weinberg V, et al. Long-term outcomes following radiation therapy for prostate cancer patients with lymph node metastases at diagnosis treated with and without surgery. *Am J Clin Oncol* 2016; 39: 167–172.
7. Lee D, Choi SK, Park J, et al. Comparative analysis of oncologic outcomes for open vs. robot-assisted radical prostatectomy in

- high-risk prostate cancer. *Korean J Urol* 2015; 56: 572–579.
8. Li X, Wang S, Li Z, et al. The lncRNA NEAT1 facilitates cell growth and invasion via the miR-211/HMGA2 axis in breast cancer. *Int J Biol Macromol* 2017; 105: 346–353.
 9. Jiang H, Zhang H, Hu X, et al. Knockdown of long non-coding RNA XIST inhibits cell viability and invasion by regulating miR-137/PXN axis in non-small cell lung cancer. *Int J Biol Macromol* 2018; 111: 623–631.
 10. Wang A, Jin C, Li H, et al. LncRNA ADAMTS9-AS2 regulates ovarian cancer progression by targeting miR-182-5p/FOXF2 signaling pathway. *Int J Biol Macromol* 2018; 120: 1705–1713.
 11. Zhu L, Zhu Q, Wen H, et al. Mutations in GAS5 affect the transformation from benign prostate proliferation to aggressive prostate cancer by affecting the transcription efficiency of GAS5. *J Cell Physiol* 2019; 234: 8928–8940.
 12. Chen Y, Wang Q, Wang M, et al. Overexpressed LRIG3 gene ameliorates prostate cancer through suppression of cell invasion and migration. *Int J Biol Macromol* 2019; 124: 1–9.
 13. Lu J, Dong W, He H, et al. Autophagy induced by overexpression of DCTPP1 promotes tumor progression and predicts poor clinical outcome in prostate cancer. *Int J Biol Macromol* 2018; 118: 599–609.
 14. Li H, Wang X, Wen C, et al. Long noncoding RNA NORAD, a novel competing endogenous RNA, enhances the hypoxia-induced epithelial-mesenchymal transition to promote metastasis in pancreatic cancer. *Mol Cancer* 2017; 16: 169.
 15. Li Q, Li C, Chen J, et al. High expression of long noncoding RNA NORAD indicates a poor prognosis and promotes clinical progression and metastasis in bladder cancer. *Urol Oncol* 2018; 36: 310.e15–310.e22.
 16. Wang L, Du L, Duan W, et al. Overexpression of long noncoding RNA NORAD in colorectal cancer associates with tumor progression. *Oncotargets Ther* 2018; 11: 6757–6766.
 17. Zhang J, Li XY, Hu P, et al. LncRNA NORAD contributes to colorectal cancer progression by inhibition of miR-202-5p. *Oncol Res* 2018; 26: 1411–1418.
 18. Wu X, Lim ZF, Li Z, et al. NORAD expression is associated with adverse prognosis in esophageal squamous cell carcinoma. *Oncol Res Treat* 2017; 40: 370–374.
 19. Miao Z, Guo X and Tian L. The long non-coding RNA NORAD promotes the growth of gastric cancer cells by sponging miR-608. *Gene* 2019; 687: 116–124.
 20. Yang Z, Zhao Y, Lin G, et al. Noncoding RNA activated by DNA damage (NORAD): biologic function and mechanisms in human cancers. *Clin Chim Acta* 2019; 489: 5–9.
 21. Kawasaki N, Miwa T, Hokari S, et al. Long noncoding RNA NORAD regulates transforming growth factor-beta signaling and epithelial-to-mesenchymal transition-like phenotype. *Cancer Sci* 2018; 109: 2211–2220.
 22. He H, Yang H, Liu D, et al. LncRNA NORAD promotes thyroid carcinoma progression through targeting miR-202-5p. *Am J Transl Res* 2019; 11: 290–299.