Journal of Cell and Molecular Research (2021) 12 (2), 49-55 DOI: 10.22067/jcmr.2021.67052.0

Research Article

LncRNA *HOXD-AS1* Is Upregulated in Ovarian Cancer

Seyedeh Nahid Fotuhi [1], Mohammad Khalaj-Kondori [1,*], Hadis Karimi [2]

[1] *Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran*
[2] *Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*

Received 6 November 2020 Accepted 12 March 2021

Abstract

Patients with ovarian cancer are mostly diagnosed at advanced stages which leads to poor prognosis and high mortality rate. Deregulation of lncRNA *HOXD-AS1* expression associates with cancer development and metastasis. However, the expression level of this lncRNA in ovarian cancer is not determined.50 paired ovarian tumors and their adjusted normal tissues were included in the study. Total RNA was extracted by TRIzol® Reagent and reverse-transcribed to cDNA using PrimeScript II cDNA synthesis kit. The expression levels of *HOXD-AS1* were quantified by qRT-PCR and compared. The Roc curve analysis was used to evaluate the capacity of *HOXD-AS1* as a biomarker for ovarian cancer. We observed that lncRNA *HOXD-AS1* was significantly upregulated in ovarian tumors compared to their adjusted normal tissues ($p<0.003$). Moreover, the ROC curve analysis revealed that the lncRNA *HOXD-AS1* expression level could discriminate tumoral and non-tumoral tissues with 85% sensitivity and 88% specificity. The lncRNA *HOXD-AS1* expression level might be considered as a potential biomarker for ovarian cancer development.

***Keywords*:** Ovarian cancer, Biomarker, lncRNA, *HOXD-AS1*, Gene expression

Introduction

Ovarian cancer (OC) can be the most lethal gynecological cancer and the seventh most common malignancy worldwide (Zhang et al., 2019). According to the researches, many countries show a high age-standardized incidence rate of ovarian cancer (Momenimovahed et al., 2019; Zhang et al., 2019). Despite this severe disease is one of the most mortality determinants in women, improved diagnostic methods have caused its incidence and mortality to be decreasing during the last few decades (La Vecchia, 2001; Razi et al., 2016). Although, there is no specific and sensitive screening method for ovarian cancer, small tumors are still the most important prognostic factors. This disease is usually diagnosed after metastasis of cancer, so the survival rate is a bit low (Knutson et al., 2015; LaDuca et al., 2019). Nowadays, molecular biomarkers are considerably growing and scientists attempt to introduce them as a diagnostic tool for severe diseases (Bignotti et al., 2006; Liu et al., 2014). Using these biomarkers leads to early-stage diagnosis which is prominent factor to improve survival (Ditto et al., 2019). In this regard, researchers are trying to discover novel diagnostic biomarkers/panels for early diagnosis of ovarian cancer. Therefore, many molecular biomarkers/panels have been recognized (Dochez et al., 2019; Zhang et al., 2011). For instance, the combination of serum *CA125* and *HE4* comes to be one of the most popular markers which have been studied (Dochez et al., 2019), but markers should possess high sensitivity and specificity (Jacobs and Bast Jr, 1989); and still need further validation.

Homeobox (HOX) genes play an essential role in embryonic development and oncogenesis. The *HOX* clusters contain various non-protein-coding RNAs, including some lncRNAs (Li et al., 2019). *HOXD-Antisense1* (*HOXD-AS1*) is encoded by the HOXD cluster on human chromosome 2q31.2 in an antisense manner (Goode et al., 2010). It is evolutionary conserved among hominids and shows all bona fide features of a gene. *HOXD-AS1* is activated by PI3K/AKT pathway and plays an important role in cell differentiation (Yarmishyn et al., 2014). Knock-down of *HOXD-AS1* confirmed that it can control the expression of clinically significant protein-coding genes which are the hallmarks of metastatic cancer. Moreover, studies

*Corresponding author's e-mail address:
khalaj@tabrizu.ac.ir, khalaj49@gmail.com

revealed that *HOXD-AS1* is usually overexpressed in hepatocellular carcinoma as well as patients with metastatic cancer (Soshnikova and Duboule, 2009; Yuan et al., 2014). *HOXD-AS1* activates a GTPase protein in the MERK/ERK signaling cascade, leading to metastasis through impeding of the apoptosis (Fang and Fullwood, 2016). Here, we compared the expression levels of *HOXD-AS1* in 50 ovarian tumors and their paired adjusted normal tissues. Moreover, the potency of HOXD-AS1 as an ovarian cancer biomarker was evaluated with ROC curve analysis.

Materials and Methods

Study subjects

50 women with ovarian cancer who had been referred to AL-Zahra Hospital of Tabriz were included in the study. After surgical operation, 50 paired ovarian tumor and marginal non-tumor samples were collected. The samples were diagnosed and approved by the pathologist. None of the patients had received chemotherapy before sampling. The study protocol was approved by the Clinical Research Ethics Committee and all subjects signed the informed consent according to the approved guidelines of AL-Zahra Hospital. Clinical information was collected from hospital records as well as by patient interviews. Table 1 represents the clinicopathologic data of the samples.

Total RNA purification

All tissue samples were collected in RNase/DNase free tubes. Samples were snap-frozen in liquid nitrogen and stored at −80°C. For RNA extraction, 30-40 mg of tissue sample was pulverized under liquid nitrogen using pestle and mortar. TRIzol® Reagent (Ambion) was used to extract total RNA from the samples according to the manufacturer's instructions. Protein and DNA contamination was eliminated by RNA Purification Kit (TIANGEN, Beijing, China). The RNA purification was evaluated by a NanoDrop ND-1000 spectrophotometer at 260 and 280 nm and the integrity of the samples was assessed by 1% gel electrophoresis.

qRT-PCR

The RNA samples were treated with DNase I according to the manufacturer's instructions (Takara, Japan). 500 ng total RNA was used for reverse transcription reaction using PrimeScript II cDNA synthesis kit (Takara, Japan). The cDNAs were applied for amplification of lncRNA *HOXD-AS1* using primers; 5′- TAATGCCAAGAACTCCCAG -3′ (forward) and 5′- GTATTCAAGGGACAGTCACAG -3′ (reverse) as well as *GAPDH* using primers; 5′- GAGAAGTATGACAACAGCCTC-3′ (forward) and 5′- TGAGTCCTTCCACGATAC -3′ (reverse) as an internal control. Quantification of the *HOXD-AS1* expression level was done by SYBR- Ampliqon (RealQ Plus 2x Master Mix Green) with an Applied Biosystems Step One Plus system using $2^{-\Delta CT}$ method (Livak and Schmittgen, 2001). The Real-time PCR reactions were run at 95°C for 10 min, followed by 40 cycles of 95°C for 20 sec, 62°C for 25 sec and 72°C for 25 sec, in 20 µl total volumes. All amplification reactions were done in duplicate format.

Statistical analysis

The data were analyzed with SPSS 24.0 (USA). The student *t*-test was used for comparison of the *HOXD-AS1* expression levels between the tumor and

Table 1. Clinicopathologic data
The clinical data are written as percentage and numbers of patients in each group for disease stage.
RMI: risk of malignancy index

	Non-tumor marginal tissue	Ovarian tumor tissue
	n = 50	n = 50
Age, years	50 (33 – 68)	50 (33 – 68)
Preoperative RMI	57 (0-1740)	4327 (33–53,000)
Disease Stage		Stage I: (n=17; 6.8%) Stage II: (n= 15; 4.9%) Stage III: (n=12; 43.9%) Stage IV: (n=6; 47.8%)
CA 125 by FIGO class: U/mL		Stage I: 66 Stage II: 229 Stage III: 910 Stage IV: 891

non-tumor control groups. Differences were considered significant at p value <0.05. Receiver-operating characteristic (ROC) curves and area under the curve (AUC) were used to assess the possibility of using *HOXD-AS1* as a diagnostic tool for detecting ovarian cancer.

Results

HOXD-AS1 expression is upregulated in ovarian cancer

The lncRNA *HOXD-AS1* expression in ovarian tumor tissues was quantified and compared with that of paired non-tumor marginal tissues by qRT-PCR. The results showed that the expression of *HOXD-AS1* in carcinoma tissues was significantly higher (p < 0.003) than those of the non-tumor marginal tissues (Figure 1A). Further correlation analysis revealed that the *HOXD-AS1* expression level was positively correlated with the disease stage (r = 0.3271, P <0.001) (Figure 1B).

HOXD-AS1 expression level might serve as a biomarker for ovarian cancer diagnosis

The biomarker capacity of the *HOXD-AS1* expression level for ovarian cancer was evaluated with the receiver operating characteristics (ROC) curve analysis. The ROC curve analysis showed an AUC (area under the curves) of 0.88 (P < 0.0001, 95% CI 0.8141- 0.9495). This analysis revealed that the *HOXD-AS1* expression level could discriminate ovarian tumor and non-tumor tissues with 85% sensitivity and 88% specificity (Figure 2A). The corresponding dot plot with the data distribution is depicted in Figure 2B.

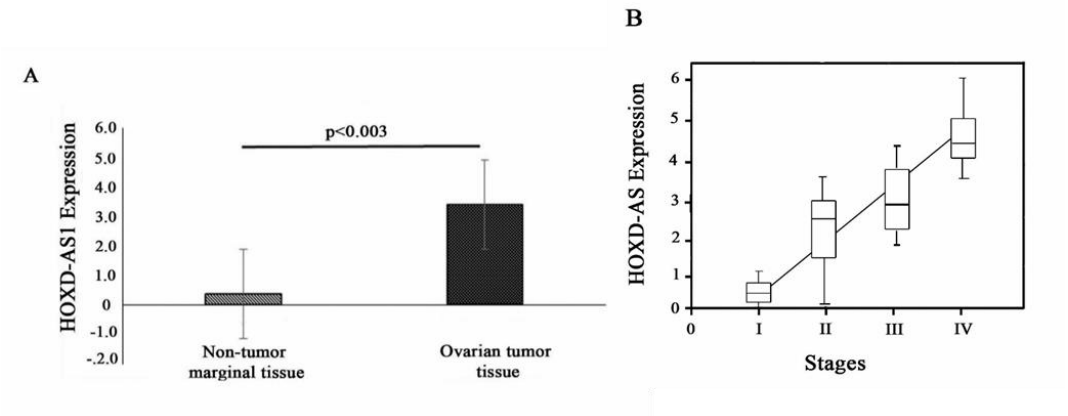

Figure 1. A) The expression of *HOXD-AS1* in carcinoma tissues was significantly higher (P< 0.003) than those of the non-tumor marginal tissues. B) The expression level shows positive correlation with disease stages.

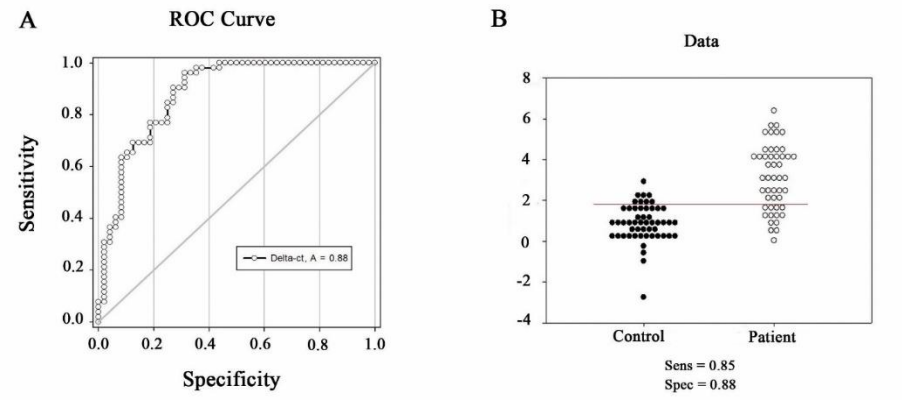

Figure 2. A) The data show that the *HOXD-AS1* expression level could discriminate ovarian tumor and non-tumor tissues with 85% sensitivity and 88% specificity. B) The corresponding dot plot showing the relative expression data distribution.

Discussion

LncRNAs are cis-/trans-regulating factors that their deregulation could result in aberrant gene expression (Chi et al., 2017). These deregulations could promote tumor development, progression, and metastasis of various type of cancers (Prensner et al., 2013; Yang et al., 2013; Yuan et al., 2014). Deregulation of the *HOXD-AS1* expression has been already reported in different cancers including bladder, cervical, colorectal, gastric, ovarian, prostate, and non-small cell lung cancers as well as hepatocellular carcinoma, melanoma, and osteosarcoma (Xie et al., 2019).

Here we compared the expression level of *HOXD-AS1* in ovarian tumor and non-tumor tissues to find out whether it may implicate with ovarian cancer development. Results showed that lncRNA *HOXD-AS1* was significantly upregulated in ovarian cancer and suggested its potential role in the ovarian cancer development. These findings are in line with the reports by Dong et al., where they observed a significant overexpression of *HOXD-AS1* in epithelial ovarian cancer tissues (Dong et al., 2019). Moreover, overexpression of this lncRNA was reported in an epithelial ovarian cancer cell line (Zhang et al., 2017). Further research has revealed that *HOXD-AS1* promotes cell proliferation, invasion, and epithelial-mesenchymal transition in epithelial ovarian cancer cells (Dong et al., 2019; Wang et al., 2018; Zhang et al., 2017), as well as invasion and metastasis in hepatocellular carcinoma cells (Wang et al., 2017).

Involving in the MEK\ERK signaling cascade (Larman et al., 2011; Yaginuma et al., 1992), *HOXD-AS1* also acts as an oncogene and changes oncogenic processes such as cell proliferation, differentiation, apoptosis, invasion, and metastasis (Wang et al., 2018; Xie et al., 2019). Consistently, we found out a positive correlation between this lncRNA expression level and the tumor stage (Figure 1B), implying that the elevated levels of *HOXD-AS1* may contribute to tumor progression. This evidence suggests that *HOXD-AS1* might be considered as a novel prognostic biomarker for malignant tumors (Dong et al., 2019).

To evaluate the biomarker capacity of this lncRNA for ovarian cancer development, we used ROC curve analysis. The results showed that the expression level of lncRNA *HOXD-AS1* could discriminate ovarian tumor and non-tumor samples with 85% sensitivity and 88% specificity. Therefore, *HOXD-AS1* expression level might serve as a potential biomarker for ovarian cancer development.

Although numerous studies have been published on the potential possibility of noncoding RNAs as cancer biomarkers (Russell et al., 2019; Sheng et al., 2020; Taylor and Gercel-Taylor, 2008), there is no certain lncRNA biomarker to diagnose ovarian cancer. However, some lncRNAs such as *MALAT1* (Zou et al., 2016), *SNHG15* (Qu et al., 2019), *ATB* (Yuan et al., 2020), *HOXA10*, and *HOXA11* (Fiegl et al., 2008; Vosseler et al., 2009) have been introduced as potential biomarkers for the ovarian cancer diagnosis.

In conclusion, this study approved that the lncRNA *HOXD-AS1* was significantly upregulated in ovarian cancer and its expression level might be considered as a potential biomarker for ovarian cancer development.

Acknowledgement

This study was supported by the research deputy of University of Tabriz. Authors appreciate all patients involved in the study.

Conflict of interest

The authors declare that they have no competing interests.

References

Bignotti E., Tassi R. A., Calza S., Ravaggi A., Romani C., Rossi E., et al. (2006) Differential gene expression profiles between tumor biopsies and short-term primary cultures of ovarian serous carcinomas: identification of novel molecular biomarkers for early diagnosis and therapy. Gynecologic oncology 103:405-416.

Chi J.-s., Li J.-z., Jia J.-j., Zhang T., Liu X.-m., and Yi, L. (2017). Long non-coding RNA ANRIL in gene regulation and its duality in atherosclerosis. Current Medical Science 37: 816-822.

Ditto A., Leone Roberti Maggiore U., Bogani G., Martinelli F., Chiappa V., Evangelista M. T., et al. (2019). Predictive factors of recurrence in patients with early-stage epithelial ovarian cancer. International Journal of Gynecology & Obstetrics 145:28-33.

Dochez V., Caillon H., Vaucel E., Dimet J., Winer N., and Ducarme G. (2019). Biomarkers and algorithms for diagnosis of ovarian cancer: CA125, HE4, RMI and ROMA, a review. Journal of ovarian research 12:28.

Dong S., Wang R., Wang H., Ding Q., Zhou X., Wang J.,et al. (2019). HOXD-AS1 promotes the epithelial to mesenchymal transition of ovarian cancer cells by regulating miR-186-5p and PIK3R3. Journal of Experimental & Clinical Cancer Research 38:110.

Fang Y., and Fullwood M. J. (2016). Roles, functions, and mechanisms of long non-coding RNAs in cancer. Genomics, proteomics & bioinformatics 14: 42-54.

Fiegl H., Windbichler G., Mueller-Holzner E., Goebel G., Lechner M., Jacobs I. J., and Widschwendter M. (2008). HOXA11 DNA methylation—a novel prognostic biomarker in ovarian cancer. International journal of cancer 123: 725-729.

Goode E. L., Chenevix-Trench G., Song H., Ramus S. J., Notaridou M., Lawrenson K., et al. (2010). A genome-wide association study identifies susceptibility loci for ovarian cancer at 2q31 and 8q24. Nature genetics 42: 874-879.

Jacobs I., and Bast Jr R. C. (1989). The CA 125 tumour-associated antigen: a review of the literature. Human reproduction 4: 1-12.

Knutson K. L., Karyampudi L., Lamichhane P., and Preston C. (2015). RETRACTED ARTICLE: Targeted immune therapy of ovarian cancer. Cancer and Metastasis Reviews 34: 53-74.

La Vecchia C. (2001). Epidemiology of ovarian cancer: a summary review. European Journal of Cancer Prevention 10: 125-129.

LaDuca H., Polley E. C., Yussuf A., Hoang L., Gutierrez S., Hart S. N.,et al. (2019). A clinical guide to hereditary cancer panel testing: evaluation of gene-specific cancer associations and sensitivity of genetic testing criteria in a cohort of 165,000 high-risk patients. Genetics in Medicine, 1-9.

Larman H. B., Zhao Z., Laserson U., Li M. Z., Ciccia A., Gakidis M. A. M., et al. (2011). Autoantigen discovery with a synthetic human peptidome. Nature biotechnology 29: 535-541.

Li L., Wang Y., Song, G., Zhang X., Gao S., and Liu H. (2019). HOX cluster-embedded antisense long non-coding RNAs in lung cancer. Cancer letters 450:14-21.

Liu R., Wang X., Aihara K., & Chen L. (2014). Early diagnosis of complex diseases by molecular biomarkers, network biomarkers, and dynamical network biomarkers. Medicinal research reviews 34, 455-478.

Livak K. J., and Schmittgen T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. methods 25:402-408.

Momenimovahed Z., Tiznobaik A., Taheri S., and Salehiniya H. (2019). Ovarian cancer in the world: epidemiology and risk factors. International journal of women's health 11:287.

Prensner J. R., Iyer M. K., Sahu A., Asangani I. A., Cao Q., Patel L., et al. (2013). The long noncoding RNA SChLAP1 promotes aggressive prostate cancer and antagonizes the SWI/SNF complex. Nature genetics 45:1392.

Qu C., Dai C., Guo Y., Qin R., and Liu J. (2019). Long noncoding RNA SNHG15 serves as an oncogene and predicts poor prognosis in epithelial ovarian cancer. OncoTargets and therapy 12, 101.

Razi S., Ghoncheh M., Mohammadian-Hafshejani A., Aziznejhad H., Mohammadian M., and Salehiniya H. (2016). The incidence and mortality of ovarian cancer and their relationship with the Human Development Index in Asia. ecancermedicalscience 10.

Russell M. R., Graham C., D'Amato A., Gentry-Maharaj A., Ryan A., Kalsi J. K.,et al. (2019). Diagnosis of epithelial ovarian cancer using a combined protein biomarker panel. British journal of cancer 121: 483.

Sheng R., Li X., Wang Z., and Wang X. (2020). Circular RNAs and their emerging roles as diagnostic and prognostic biomarkers in ovarian cancer. Cancer Letters 473:139-147.

Soshnikova N., and Duboule D. (2009). Epigenetic temporal control of mouse Hox genes in vivo. Science 324: 1320-1323.

Taylor D. D., and Gercel-Taylor C. (2008). MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecologic oncology 110:13-21.

Vosseler S., Lederle W., Airola K., Obermueller E., Fusenig, N. E., and Mueller M. M. (2009). Distinct progression-associated expression of tumor and stromal MMPs in HaCaT skin SCCs correlates with onset of invasion. International journal of cancer 125: 2296-2306.

Wang H., Huo X., Yang X.-R., He,J., Cheng,L., Wang N.,et al. (2017). STAT3-mediated upregulation of lncRNA HOXD-AS1 as a ceRNA facilitates liver cancer metastasis by regulating SOX4. Molecular cancer 16: 136.

Wang Y., Zhang W., Wang Y., and Wang S. (2018). HOXD-AS1 promotes cell proliferation, migration and invasion through miR-608/FZD4 axis in ovarian cancer. American journal of cancer research 8: 170.

Xie S., Yang Y., Zhang J., Zhou S., Xie S., and Hua Y. (2019). HOXD-AS1: a novel oncogenic long intergenic non-coding RNA in humans. European review for medical and pharmacological sciences 23: 2898-2907.

Yaginuma Y., Yamashita K., Kuzumaki N., Fujita M., and Shimizu T. (1992). ras oncogene product p21 expression and prognosis of human ovarian tumors. Gynecologic oncology 46: 45-50.

Yang L., Lin C., Jin C., Yang J. C., Tanasa B., Li W., et al. (2013). lncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs. Nature 500: 598-602.

Yarmishyn A. A., Batagov A. O., Tan J. Z., Sundaram G. M., Sampath P., Kuznetsov V. A., and Kurochkin I. V. (2014). HOXD-AS1 is a novel lncRNA encoded in HOXD cluster and a marker of neuroblastoma progression revealed via integrative analysis of noncoding transcriptome. BMC genomics 15: S7.

Yuan D., Zhang X., Zhao Y., Qian H., Wang H., He C., et al. (2020). Role of lncRNA-ATB in ovarian cancer and its mechanisms of action. Experimental and Therapeutic Medicine 19: 965-971.

Yuan J.-h., Yang F., Wang F., Ma J.-z., Guo Y.-j., Tao Q.-f., et al. (2014). A long noncoding RNA activated by TGF-β promotes the invasion-metastasis cascade in hepatocellular carcinoma. Cancer cell 25: 666-681.

Zhang B., Cai F. F., and Zhong X. Y. (2011). An overview of biomarkers for the ovarian cancer diagnosis. European Journal of Obstetrics & Gynecology and Reproductive Biology 158: 119-123.

Zhang Y., Dun Y., Zhou S., and Huang X.-H. (2017). LncRNA HOXD-AS1 promotes epithelial ovarian cancer cells proliferation and invasion by targeting miR-133a-3p and activating Wnt/β-catenin signaling pathway. Biomedicine & Pharmacotherapy 96: 1216-1221.

Zhang Y., Luo G., Li M., Guo P., Xiao Y., Ji H., and Hao Y. (2019). Global patterns and trends in ovarian cancer incidence: age, period and birth cohort analysis. BMC cancer 19: 984.

Zou A., Liu R., and Wu X. (2016). Long non-coding RNA MALAT1 is up-regulated in

ovarian cancer tissue and promotes SK-OV-3 cell proliferation and invasion. Neoplasma 63, 865.