LncRNA HOXD-AS1 Is Upregulated in Ovarian Cancer

Seyyedeh 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

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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 4q31.2 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
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 IncRNA HOXD-AS1 using primers; 5′- TAATGCCAAGAACTCCCAG -3′ (forward) and 5′- GTATTCAAGGGACAGTCACAG -3′ (reverse) as well as GAPDH using primers; 5′- GAGAAGTATGACAACGCTC-3′ (forward) and 5′- TGAGTCCTTCCAGCATAC-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−ΔΔCT 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).

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

The student t-test was used for comparison of the HOXD-AS1 expression levels between the tumor and normal tissues.

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

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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 IncRNA 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](http://jcmr.um.ac.ir)

**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](http://jcmr.um.ac.ir)

**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 IncRNA 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 IncRNA 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 IncRNA 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 IncRNA for ovarian cancer development, we used ROC curve analysis. The results showed that the expression level of IncRNA 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 IncRNA biomarker to diagnose ovarian cancer. However, some IncRNAs such as MALAT1 (Zou et al., 2016), SNHG15 (Qu et al., 2019), ATB (Yuan et al., 2020), HOXA10, and HOXA11 (Fieggl 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 IncRNA HOXD-AS1 was significantly upregulated in ovarian cancer and its expression level might be considered as a potential biomarker for ovarian cancer development.

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Conflict of interest
The authors declare that they have no competing interests.

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

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