Experimental Cell Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Knockdown of MALAT1 enhances chemosensitivity of ovarian cancer cells to cisplatin through inhibiting the Notch1 signaling pathway

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ARTICLE INFO

Keywords:
MALAT1
Notch1
ABCC1
Cisplatin
Chemosensitivity
Ovarian cancer

ABSTRACT

Long non-coding RNAs (lncRNAs) are critical regulators in chemoresistance of various tumors including ovarian cancer. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has been reported to be upregulated and contributed to ovarian cancer tumorigenesis. The aim of this study was to explore the roles of MALAT1 and the underlying molecular regulatory mechanism in the chemoresistance of ovarian cancer cells. Our data demonstrated that MALAT1 and Notch1 mRNA were upregulated in ovarian cancer tissues, as well as cisplatin (CDDP)-resistant ovarian cancer cells. A positive correlation between MALAT1 and Notch1 mRNA expression was observed. MALAT1 knockdown significantly attenuated CDDP resistance, and enhanced CDDP-induced apoptosis in CDDP-resistant ovarian cancer cells. MALAT1 knockdown enhanced CDDP-induced apoptosis *in vivo*, as indicated by upregulation of Bax protein expression and downregulation of Bcl-2 protein expression. Additionally, MALAT1 knockdown inhibited the Notch1 pathway and ABCC1 expression in CDDP-resistant ovarian cancer cells. MALAT1 was demonstrated to interact with Notch1. Notch1 knockdown attenuated CDDP resistance, and downregulated the protein expression of ABCC1 in ovarian cancer cells. Taken together, our findings suggested that knockdown of MALAT-1 enhanced chemosensitivity of ovarian cancer cells to CDDP through inhibiting Notch1 signaling pathway.

1. Introduction

Ovarian cancer is one of the most prevalent gynecological malignant tumors with an estimated 200,000 new diagnosed cases every year, accounting for the high rates of morbidity and mortality [1,2]. The 5-year survival rate for stage I or II ovarian cancer is 80–95%, whereas diagnosis at more advanced stages is less than 30% [3]. The most current chemotherapeutic combination used in ovarian cancer treatment is carboplatin combined with paclitaxel. But, the therapeutic effects are unsatisfactory. Platinum based-chemotherapy is used as one of the typical first-line neoadjuvant for advanced ovarian cancer following surgical resection of visible nidus [4]. Cisplatin (CDDP), the most common platinum-based compound, is currently one of the common chemotherapeutic drugs against ovarian cancer [5]. However, the therapeutic effectiveness of CDDP-based therapeutic regimen is limited due to the progressive development of multidrug resistance (MDR) during the chemotherapy progress, which is a major obstacle in the clinical treatment of ovarian cancer patients [6]. Ovarian cancer MDR involves multiple drug-resistant molecules and complex mechanisms, such as ATP-binding cassette (ABC) transporter family, apoptosis induction, autophagy induction, cancer stem cell regulation, miRNA regulation, lncRNA regulation, hypoxia induction, DNA damage and repair, and epigenetic regulation [7]. As a major mechanism of MDR, overexpression of ABC membrane transporters, such as P-glycoprotein (P-gp) and resistance-related protein (ABCC1/MRP1), has been identified as the major determinant of chemoresistance in cancer cells [8]. ABCC1 is able to transferred chemotherapeutic agents and metabolites out of cancer cells to protect them from damage. These transport properties of ABCC1 are responsible for the drug resistance in ABCC1-overexpressing cancers [9]. Overexpression of ABCC1 is responsible for the resistance to platinum drugs, including CDDP [10]. However, the upstream regulation of ABCC1 in chemotherapeutic resistance of cancer is still unknown. Therefore, it is particularly urgent to make clear the molecular mechanisms underlying the development of CDDP resistance, and develop new effective strategies for the treatment of patients with refractory ovarian cancer.

Long non-coding RNAs (lncRNAs) are generally defined as non-protein coding transcripts that are longer than 200 nucleotides. LncRNAs have been demonstrated to regulate gene expression at multiple levels, including epigenetic modification, transcriptional, and post-transcriptional processing [11]. In addition, a growing body of evidence has suggested that lncRNAs exerted their biological activities

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https://doi.org/10.1016/j.yexcr.2018.03.014
Received 25 December 2017; Received in revised form 9 March 2018; Accepted 12 March 2018

through multiple biological mechanisms, such as genomic imprinting, chromatin remodeling, miRNA-lncRNA and lncRNA-protein interaction [12]. Dysregulated lncRNAs have been proposed to be implicated in the occurrence and development of malignancies by regulating cell growth, apoptosis, differentiation, and cell cycle of tumor cells [13]. Interestingly, an increasing body of evidence has also suggested that lncRNAs contribute to the chemoresistance of a variety of tumors including ovarian cancer [14,15]. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1), located on chromosome 11q13, is the firstly discovered cancer-associated lncRNA. MALAT-1 is upregulated in patients at high risk for metastasis of non-small-cell lung tumors (NSCLC) [16]. Also, MALAT1 has been found to be highly expressed in various tumors, such as lung cancer, colon cancer, cervix cancer, and prostate cancer, which can be an independent prognostic factor for survival [17]. Previously, MALAT1 was reported to be upregulated in ovarian cancer and function as an important contributor to ovarian cancer tumorigenesis and development [18–20]. However, the role of MALAT1 in the development of CDDP resistance in ovarian cancer cells is still largely unknown.

Recently, increasing studies have focused on the important role of the Notch signaling pathway, which is a novel mechanism for carcinogenesis [21]. The binding of Notch transmembrane receptors to its ligand in canonical Notch signaling results in the proteolytic cleavage by γ-secretase, and then the released Notch1 intracellular domain is translocated to the nucleus and drives the expression of multiple targets including Hes/Hey families [22]. Notch1 is one of the highly conserved Notch family members, which plays important roles in biological processes including proliferation and apoptosis [22]. Dysregulation of Notch1 signaling has documented to be involved in the development and progression of human malignancies, including ovarian cancer [23,24]. It was reported that inhibition of Notch1 pathway increased chemosensitivity of CDDP-resistant ovarian cancer cells to CDDP [25]. Whether the molecular mechanism of MALAT1-induced CDDP resistance of ovarian cancer cells is mediated by the Notch1 pathway remains to be explored.

In the present study, we explored the effects of MALAT1 on the CDDP resistance and CDDP-induced apoptosis of CDDP-sensitive and CDDP-resistant ovarian cancer cells and its molecular mechanism.

2. Materials and methods

2.1. Patients and samples

Twenty paired tumor tissue samples and adjacent normal tissue samples (1 cm away from the tumor margin) were obtained from patients with ovarian cancer undergoing surgery in the Department of Obstetrics and Gynecology, The First People's Hospital of Shangqiu between 2014 and 2016. The primary ovarian cancer was histopathologically diagnosed according to the International Federation of Obstetrics and Gynecology (FIGO) criteria. None of the ovarian cancer patients received adjuvant therapies including radiotherapy, chemotherapy or hormonal therapy prior to surgery. Clinicopathological features of patients with ovarian cancer were shown in Table 1. All tissues were rapidly frozen in liquid nitrogen and immediately stored at −80 °C for further analysis. The study protocols were approved by the ethics committee of The First People's Hospital of Shangqiu and written informed consent was obtained from each participant.

2.2. Cell culture and transfection

The CDDP-sensitive ovarian cancer cell lines (A2780, OVCAR3 and COC1) and their CDDP-resistant ovarian cancer cell lines (A2780/CDDP, COC1/CDDP, OVCAR3/DDP) were used in our study. OVCAR3 and OVCAR3/DDP cells were purchased from Shanghai Institute of Cell Biology, China Academy of Sciences (Shanghai, China). COC1, COC1/CDDP, A2780, and A2780/CDDP cells and normal ovarian cancer surface epithelial cell line (IOSE 364) were obtained from China Center for Type Culture Collection (Wuhan, China). These cells were cultured in RPMI-1640 culture medium (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc., Carlsbad, CA, USA), 1% penicillin/streptomycin (Hyclone, Logan, UT, USA) at 37 °C in a humidified atmosphere containing 5% CO_2. To ensure maintenance of the CDDP resistance phenotypes, A2780/CDDP, OVCAR3/CDDP and COC1/CDDP cells were routinely incubated with 1 μM CDDP (Sigma-Aldrich, St Louis, MO, USA).

To knock down MALAT1 expression, short hairpin (shRNA) oligonucleotide of MALAT1 (sh-MALAT1) and one non-target oligonucleotide (negative control shRNA, sh-NC) were cloned into the pGPU6/GFP/Neo vectors (GenePharma, Shanghai, China), respectively. After cloning, amplification, and DNA sequencing, these vectors were transfected into ovarian cancer cells. To overexpress MALAT1, the recombined plasmid (pcDNA-MALAT1) was constructed by inserting the whole MALAT1 sequence into pCDNA3.1 vector (Invitrogen, Carlsbad, CA, USA). siRNA against Notch1 (si-Notch1) and siRNA scrambled control (si-NC) were purchased from GenePharma (Shanghai, China). Cell transfection was performed using Lipofectamine 2000 (Invitrogen). Cells were collected at 48 h post-transfection for next experiments.

Table 1
Clinicopathological features of patients with ovarian cancer.

Characteristics	Number of cases
Age (years)	
≤50	8
> 50	12
Tumor size	
≤5 cm	9
> 5 cm	11
Stage	
I	3
II	5
III	8
IV	4
Histology type	
Serous adenocarcinoma	12
Mucoid adenocarcinoma	5
Endometrioid carcinoma	3
Pathological grade	
1	4
2	7
3	9
Lymph node metastasis	
Negative	13
Positive	7

2.3. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis

Total RNA from the tissues and cells was extracted using TRIzol reagent (Invitrogen). RNA quality and concentration were quantified using a Nanodrop 2000 system (Thermo Fisher Scientific, Inc.). For the detection of MALAT1 and Notch1 mRNA, PrimeScript RT reagent kit (TaKaRa Bio, Shiga, Japan) was used to synthesize cDNA from 1 μg of total RNA. Then, RT-PCR was performed using a SYBR Premix Ex Taq™ kit (TaKaRa Bio) on an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). β-actin expression was used as the internal control. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences were as follows: MALAT1 forward, 5′-GAC CCT TCA CCC CTC ACC-3′ and reverse, 5′-TTA TGG ATC ATG CCC ACA AG-3′; Notch1 forward, 5′-TGT TAA TGA GTG CAT CTC CAA-3′ and reverse, 5′-CAT TCG TAG CCA TCA ATC TTG TCC-3′; β-actin forward, 5′-GCG GGA AAT CGT GCG TGA C-3′ and reverse, 5′-GGA AGG AAG GCT GGA AGA G-3′.