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Increased expression of long noncoding RNA HMMR-AS1 in epithelial ovarian cancer: an independent prognostic factor

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Abstract. – OBJECTIVE: Evidence has indicated that long noncoding RNA (lncRNAs) may have significant roles in cancer. In this study, we aimed to investigate the expression pattern and prognostic value of a noncoding RNA named as HMMR antisense RNA 1 (HMMR-AS1) in epithelial ovarian cancer (EOC).

PATIENTS AND METHODS: Differences in the expression of HMMR-AS1 between EOC and matched normal tissues were analyzed using RT-PCR. The correlation between HMMR-AS1 levels and the clinicopathological factors of the EOC patients was analyzed by x^2-test. Kaplan-Meier analysis and Cox proportional hazards regression models were explored to reveal the correlations of HMMR-AS1 expression with survival of patients.

RESULTS: HMMR-AS1 was significantly upregulated in human EOC tissues compared with adjacent normal tissues ($p < 0.01$). Clinicopathologic analysis revealed that high expression of HMMR-AS1 was associated with advanced FIGO stage ($p = 0.013$) and positive lymphatic metastasis ($p = 0.010$). Moreover, patients with higher HMMR-AS1 expression displayed shorter overall survival time ($p = 0.0075$) and progression-free survival time ($p = 0.0013$) than those with lower HMMR-AS1 expression. More importantly, multivariate analysis suggested that high expression of HMMR-AS1 was an independent prognostic indicator for EOC patients.

CONCLUSIONS: Our data suggested that HMMR-AS1 may be considered a novel prognostic factor in EOC and a specific diagnostic indicator for patients with EOC.

Key Words:
LncRNA, HMMR-AS1, Epithelial ovarian cancer, Prognosis.

Introduction

Ovarian cancer is one of the most common causes of death from all cancers among women with high morbidity and mortality rates[1,2]. Epithelial ovarian cancer (EOC) is the most common type of ovarian cancer. It is reported that ovarian cancer is associated with multistep changes in the genome[3]. Despite the significant advances in the research and treatment, the majority of patients are diagnosed with advanced disease, and the five-year survival rate for EOC is about 30%[4,5]. In China, clinical outcomes vary significantly between patients and can be difficult to predict. Therefore, it is necessary to search for novel markers for EOC, which can accurately identify the biological characteristics of tumors, enhance cancer detection and predict clinical outcome. Long noncoding RNAs (lncRNAs) are a diverse set of transcripts, which are defined as encompassing more than 200 nucleotides[6]. Although lncRNAs have no protein coding capacity, lncRNAs play functional roles in many biological processes such as cell development, proliferation, metastasis, angiogenesis, cell cycle, differentiation, invasion and migration[7,8]. In human cancers, there is growing evidence that lncRNAs are involved in cancer cell proliferation, invasion and apoptosis by regulating oncogenes and/or tumor suppressor genes[9,10]. Of note, several important functional lncRNAs have been well investigated in various including EOC, such as lncRNA HOTAIR[11], lncRNA HOXA11-AS[12], and lncRNA DUXAP10[13]. However, there are still many lncRNAs whose expression and function remain unknown. HM-

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MR antisense RNA 1(HMMR-AS1), a novel dysregulated lncRNA located at 5p34, is the antisense transcript of hyaluronan-mediated motility receptor (HMMR). Recently, upregulated expression of HMMR-AS1 and its oncogenic role have been reported in breast cancer and glioblastoma[14,15]. However, whether HMMR-AS1 was dysregulated in EOC, and its function as well as clinical significance in EOC have not been investigated. In this study, for the first time, we reported the possibility of HMMR-AS1 as a novel diagnostic and prognostic biomarker for EOC patients.

Patients and Methods

Patients

EOC and matched adjacent non-tumor tissues were obtained from 152 patients who underwent surgery at the Hebei General Hospital between September 2013 and February 2014. Tissue samples were immediately stored in liquid nitrogen for RNA extraction. None of these EOC patients had received other therapeutic treatments prior to surgery. All histological diagnoses for EOC and matched normal tissues were reviewed and recognized by three pathologists. Patients signed an informed consent and their clinical information is summarized in Table I. Prior informed consent was obtained, and the study protocol was approved by the Ethics Committee of Hebei General Hospital.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from EOC tissues using the TRIzol reagent according to the protocol and was quantified with NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA (500 ng) was reverse transcribed in a final volume of 10 mL using random primers under standard conditions for the PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). Quantitative Real-time PCR (qPCR) was performed using TaqMan Universal Master Mix II (ABI) with a 7300 Real-Time PCR System (ABI). PCR was performed with the following thermocycling conditions: an initial of 3 min at 95°C, followed by 40 cycles of 95°C for 10 s, 60°C for 30 s. The relative expression of HMMR-AS1 was calculated and normalized using the $2^{-\Delta\Delta Ct}$ method relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Each sample was analyzed in triplicate pattern. The primer sequences used in this study are shown in Table II.

Table I. Correlation of HMMR-AS1 expression with clinicopathological features of EOC.

Variables	No. of cases	HMMR-AS1 expression		*p*-value
		High	Low	
Age (years)				NS
≤ 55	73	41	32	
> 55	79	34	45	
Size (cm)				NS
≤ 5	89	41	48	
> 5	63	34	29	
FIGO stage				0.013
I-II	96	40	56	
III-IV	56	35	21	
Poor histologic differentiation				NS
Yes	55	31	24	
No	97	44	53	
Vascular invasion				NS
Yes	49	27	22	
No	103	48	55	
Lymphatic metastasis				0.010
Yes	46	30	16	
No	106	45	61	
Distant metastasis				NS
Yes	47	27	20	
No	105	48	57	

Table II. Sequence of the primers used in this study.

Genes	Primer sequences (5′-3′)
HMMR-AS1: Forward	AACTCGCCTATTTAGCCTGGG
HMMR-AS1: Reverse:	ATACCAGGAACCAGGAGTTGTGT
GAPDH: Forward	CGGAGTCAACGGATTTGGTCGTAT
GAPDH: Reverse:	AGCCTTCTCCATGGTGGTGAAGAC

Statistical Analysis

Statistical analyses were performed using Prism 7 (GraphPad, La Jolla, CA, USA) and the SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA). The comparison of the expression levels of HMMR-AS1 between EOC tissues and adjacent normal tissues were performed using the two-sample Student's *t*-test. x^2-test was used to analyze the relationship between HMMR-AS1 expression levels and the clinicopathological characteristics. Survival curves were plotted using the Kaplan-Meier method and compared using the log-rank test. The significance of survival variables was evaluated using a multivariate Cox proportional hazards regression analysis. A $p < 0.05$ was considered statistically significant.

Results

HMMR-AS1 is Highly Expressed in EOC Tissues

Firstly, we performed RT-PCR to explore whether HMMR-AS1 was dysregulated in EOC patients. As shown in Figure 1, as compared to matched normal tissues, HMMR-AS1 expression was highly expressed in EOC tissues ($p < 0.01$). Those data indicated that HMMR-AS1 might play an oncogenic role in EOC progression.

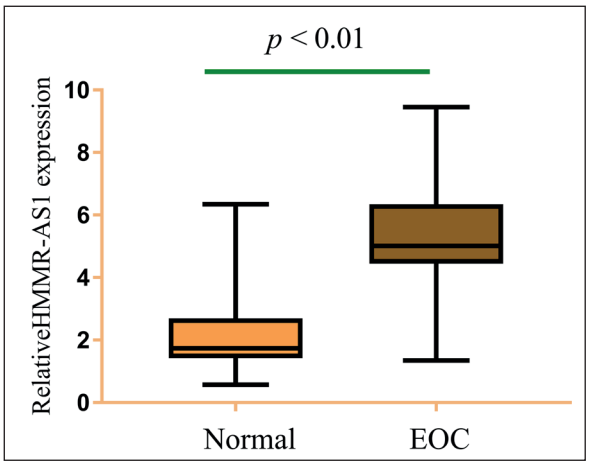

Figure 1. HMMR-AS1 was upregulated in EOC tissues. Tumor tissues and adjacent normal tissues were extracted from 152 EOC patients. The expression of HMMR-AS1 was compared by qRT-PCR.

Correlation of HMMR-AS1 Expression with Clinicopathological Factors of EOC Patients

Then, we explored the clinical association between HMMR-AS1 expression and clinicopathologic factors of EOC patients. The median value of HMMR-AS1 in all EOC tissues was 2.65 and was used as a cutoff value, and all patients were divided into two groups high-HMMR-AS1 expression group (n = 75) and low-HMMR-AS1 expression group (n = 77). As shown in Table I, we found that high expression of HMMR-AS1 was positively associated with FIGO stage ($p = 0.013$) and lymphatic metastasis ($p = 0.010$) in EOC patients. However, there were no significant associations of HMMR-AS1 expression with patients' age and size, poor histologic differentiation, vascular invasion and distant metastasis ($p < 0.05$). Thus, our results indicated that HMMR-AS1 may be a functional lncRNA in clinical progression of EOC.

Prognostic Values of HMMR-AS1 Expression in EOC

To further investigate the correlation of HMMR-AS1 expression with the prognosis of EOC patients, Kaplan-Meier analyses were performed. As shown in Figure 2 and 3, patients with higher expression of HMMR-AS1 showed significantly shorter overall survival (OS) ($p = 0.075$) and progression-free survival (PFS) ($p = 0.013$) than those with lower expression, suggesting that HMMR-AS1 might be associated with prognosis. Then, we further explored whether HMMR-AS1 could be an independent prognostic factor for EOC patients. Univariate analysis showed that FIGO stage, lymphatic metastasis and HMMR-AS1 expression were associated with both OS and PFS of EOC patients (Table III and IV). Further multivariate analysis confirmed that HMMR-AS1

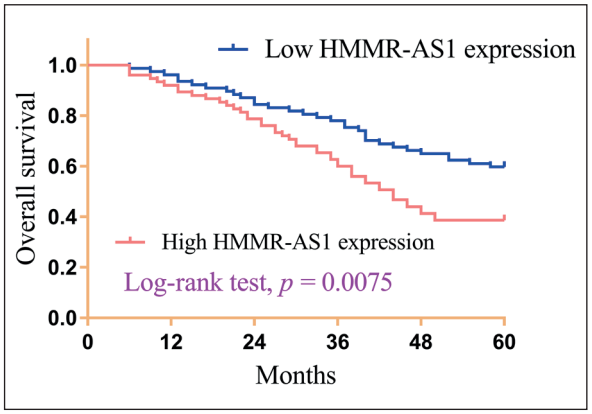

Figure 2. Kaplan-Meier survival curves of patients with EOC based on HMMR-AS1 expression level. Patients in the high expression group had significantly poorer overall survival than those in low expression group (p = 0.0075, log-rank test).

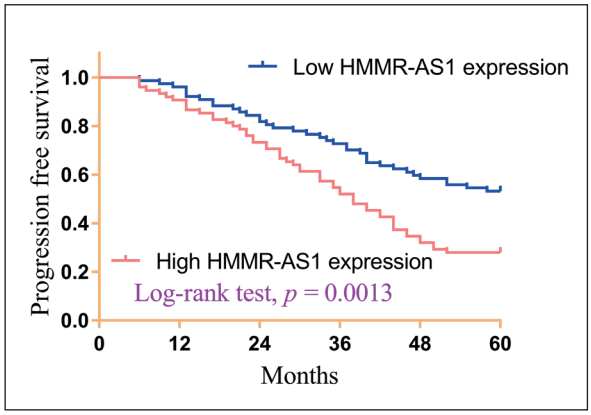

Figure 3. Kaplan-Meier survival curves of patients with EOC based on HMMR-AS1 expression level. Patients in the high expression group had significantly poorer progression-free survival than those in low expression group (p = 0.0013, log-rank test).

expression was an independent prognostic factor for both OS (HR = 3.233, 95% CI: 1.232-4.564, p = 0.018) and PFS (HR = 3.652, 95% CI: 1.169-4.732, p =0.009) of EOC patients (Table III and IV). Taken together, our results revealed that HMMR-AS1 may be a novel prognostic biomarker for EOC patients.

Discussion

In China, EOC accounted for 52000 new patients and 22000 deaths in 2017, which is the highest mortality rate among gynecologic cancers[16]. Prediction of prognosis of EOC patients is very important for the management of EOC treatments[17]. Up to date, several clinicopathologic features have been the standard for determining the clinical outcome of EOC patients, and several molecules and genetic alterations have also been reported as potential markers[18-20]. However, satisfactory prognostic methods for EOC have not been achieved. With the development of microchip analytical procedures, lncRNAs become ideal candidates for its dysregulation and critical roles in progression of EOC[21,22]. In addition, researches reported that lncRNAs can be used as a potential biomarker for the prognosis of an individuals' disease as well as a guide to determine what is the most appropriate therapy[23,24]. However, a large number of lncRNAs remain to be elucidated. Dysregulation of lncRNAs was frequently reported in EOC and its forced expression could suppress or promote tumor progression, thus suggesting the potential application of lncRNAs as diagnostic biomarkers and treatments targeting[25,26]. For instance, lncRNA NEAT1, a well-studied lncRNA in various tu-

Table III. Univariate and multivariate analysis of clinicopathological factors for overall survival.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age ≤ 55 *vs.* > 55	1.477	0.658-2.213	0.327	-	-	-
Size ≤ 5 *vs.* > 5	1.846	0.733-2.543	0.179	-	-	-
FIGO stage I-II *vs.* III-IV	3.667	1.563-5.328	**0.008**	3.015	1.126-4.164	**0.019**
Poor histologic differentiation Yes *vs.* No	1.643	0.893-2.336	0.139	-	-	-
Vascular invasion Yes *vs.* No	1.832	0.654-2.347	0.115	-	-	-
Lymphatic metastasis Yes *vs.* No	3.699	1.473-5.664	**0.005**	3.126	1.177-4.673	**0.009**
Distant metastasis Yes *vs.* No	1.546	0.932-2.137	0.127	-	-	-
HMMR-AS1 expression High *vs.* Low	3.842	1.547-5.327	**0.007**	3.233	1.232-4.564	**0.018**

Table IV. Univariate and multivariate analysis of clinicopathological factors for progression free survival.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age ≤ 55 *vs.* > 55	1.564	0.781-2.155	0.237	-	-	-
Size ≤ 5 *vs.* > 5	1.632	0.822-2.438	0.134	-	-	-
FIGO stage I-II *vs.* III-IV	3.543	1.427-5.675	**0.005**	3.223	1.267-4.645	**0.014**
Poor histologic differentiation Yes *vs.* No	1.547	0.732-2.455	0.238	-	-	-
Vascular invasion Yes *vs.* No	1.642	0.834-2.543	0.124	-	-	-
Lymphatic metastasis Yes vs. No	3.943	1.655-5.932	**0.003**	3.452	1.321-5.128	**0.006**
Distant metastasis Yes *vs.* No	1.437	0.822-2.257	0.144	-	-	-
HMMR-AS1 expression High *vs.* Low	4.153	1.568-5.776	**0.001**	3.652	1.169-4.732	**0.009**

mors, was reported to be upregulated in ovarian cancer and be associated with poor prognosis. Functionally, lncRNA NEAT1 was confirmed to promote cell proliferation and migration through sponging miR-506 in high-grade serous ovarian cancer[27]. Other lncRNAs, such as lncRNA GAS5[28], lncRNA TUBA4B[29], and lncRNA H19[30], were also reported to be functional regulators in EOC behavior and serve as potential prognostic biomarkers. HMMR-AS1, as a newly identified lncRNA, was firstly reported to be dysregulated in breast cancer and glioblastoma. In glioblastoma, knockdown of HMMR-AS1 inhibited cell migration and invasion[16]. In breast cancer, HMMR-AS1 was found to act as a tumor promoter[15]. These results highlighted the possibility of HMMR-AS1 as a potential important targeting for tumors. However, to our best knowledge, the roles of HMMR-AS1 on EOC progression have not been reported. In this study, in order to explore whether HMMR-AS1 was dysregulated in EOC patients, we collected EOC tissues and matched normal tissues from 152 EOC patients from our hospital, and performed RT-PCR: we found that HMMR-AS1 expression was significantly upregulated in EOC tissues. This trend of expression was similar with previous results in breast cancer and glioblastoma. Then, we further study the clinical significance of HMMR-AS1 by analyzing relationship between clinicopathological characteristics and HMMR-AS1 expression, finding that high HMMR-AS1 expression was significantly associated with advanced FIGO stage and positive lymphatic metastasis, indicating that HMMR-AS1 may act as a positive regulator in progression of EOC. Moreover, we performed Kaplan-Meier analyses to further demonstrate the prognosis value of HMMR-AS1 in EOC and the results indicated that the OS/PFS for patients in the high HMMR-AS1 expression group was significantly lower than that of the low HMMR-AS1 expression group. More importantly, Cox proportional regression analysis indicated that HMMR-AS1 was an independent prognostic factor for the EOC patients. However, one of the limitations of this study is that the sample size is relatively small. Further studies on a great number of patients are required to confirm our findings.

Conclusions

We showed that high expression of HMMR-AS1 is an independent factor for poor survival in newly diagnosed patients with EOC, and targeting HMMR-AS1 may be of benefit to EOC patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.
2) Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.
3) Kwa M, Muggia F. Ovarian cancer: a brief historical overview of intraperitoneal trials. Ann Surg Oncol 2014; 21: 1429-1434.
4) Teo MC. Update on the management and the role of intraperitoneal chemotherapy for ovarian cancer. Curr Opin Obstet Gynecol 2014; 26: 3-8.
5) Elzek MA, Rodland KD. Proteomics of ovarian cancer: functional insights and clinical applications. Cancer Metastasis Rev 2015; 34: 83-96.

6) Gloss BS, Dinger ME. The specificity of long noncoding RNA expression. Biochim Biophys Acta 2016; 1859: 16-22.

7) Leone S, Santoro R. Challenges in the analysis of long noncoding RNA functionality. FEBS Lett 2016; 590: 2342-2353.

8) Schmitz SU, Grote P, Herrmann BG. Mechanisms of long noncoding RNA function in development and disease. Cell Mol Life Sci 2016; 73: 2491-2509.

9) Huarte M. The emerging role of lncRNAs in cancer. Nat Med 2015; 21: 1253-1261.

10) Cheetham SW, Gruhl F, Mattick JS, Dinger ME. Long noncoding RNAs and the genetics of cancer. Br J Cancer 2013; 108: 2419-2425.

11) Bhan A, Mandal SS. LncRNA HOTAIR: a master regulator of chromatin dynamics and cancer. Biochim Biophys Acta 2015; 1856: 151-164.

12) Richards EJ, Permuth-Wey J, Li Y, Chen YA, Coppola D, Reid BM, Lin HY, Teer JK, Berchuck A, Birrer MJ, Lawrenson K, Monteiro AN, Schildkraut JM, Goode EL, Gayther SA, Sellers TA, Cheng JQ. A functional variant in HOXA11-AS, a novel long non-coding RNA, inhibits the oncogenic phenotype of epithelial ovarian cancer. Oncotarget 2015; 6: 34745-34757.

13) Zhang Q, Wang WW, Xu TH, Xu ZF. Highly expressed long non-coding RNA DUXAP10 promotes proliferation of ovarian cancer. Eur Rev Med Pharmacol Sci 2018; 22: 314-321.

14) Liu W, Ma J, Cheng Y, Zhang H, Luo W, Zhang H. HMMR antisense RNA 1, a novel long noncoding RNA, regulates the progression of basal-like breast cancer cells. Breast Cancer (Dove Med Press) 2016; 8: 223-229.

15) Li J, Ji X, Wang H. Targeting long noncoding RNA HMMR-AS1 suppresses and radiosensitizes glioblastoma. Neoplasia 2018; 20: 456-466.

16) Mao W, Tang S, Zhu Y, Xie Z, Chen W. Financial burden of healthcare for cancer patients with social medical insurance: a multi-centered study in urban China. Int J Equity Health 2017; 16: 180.

17) Jackson SE, Chester JD. Personalised cancer medicine. Int J Cancer 2015; 137: 262-266.

18) Yoshikawa N, Kajiyama H, Mizuno M, Shibata K, Kawai M, Nagasaka T, Kikkawa F. Clinicopathologic features of epithelial ovarian carcinoma in younger vs. older patients: analysis in Japanese women. J Gynecol Oncol 2014; 25: 118-123.

19) Mukhopadhyay A, Plummer ER, Elattar A, Soohoo S, Uzir B, Quinn JE, McCluggage WG, Maxwell P, Aneke H, Curtin NJ, Edmondson RJ. Clinicopathological features of homologous recombination-deficient epithelial ovarian cancers: sensitivity to PARP inhibitors, platinum, and survival. Cancer Res 2012; 72: 5675-5682.

20) Penzvalto Z, Surowiak P, Gyorffy B. Biomarkers for systemic therapy in ovarian cancer. Curr Cancer Drug Targets 2014; 14: 259-273.

21) Grammatikakis I, Panda AC, Abdelmohsen K, Gorospe M. Long noncoding RNAs (lncRNAs) and the molecular hallmarks of aging. Aging (Albany NY) 2014; 6: 992-1009.

22) Kapusta A, Feschotte C. Volatile evolution of long noncoding RNA repertoires: mechanisms and biological implications. Trends Genet 2014; 30: 439-452.

23) Wang YS, Ma LN, Sun JX, Liu N, Wang H. Long non-coding RNA CPS1-IT1 is a positive prognostic factor and inhibits epithelial ovarian cancer tumorigenesis. Eur Rev Med Pharmacol Sci 2017; 21: 3169-3175.

24) Xu QF, Tang YX, Wang X. LncRNA EBIC promoted proliferation, metastasis and cisplatin resistance of ovarian cancer cells and predicted poor survival in ovarian cancer patients. Eur Rev Med Pharmacol Sci 2018; 22: 4440-4447.

25) Li J, Yu H, Xi M, Lu X. Long noncoding RNA C17orf91 is a potential prognostic marker and functions as an oncogene in ovarian cancer. J Ovarian Res 2016; 9: 49.

26) Akrami R, Jacobsen A, Hoell J, Schultz N, Sander C, Larsson E. Comprehensive analysis of long non-coding RNAs in ovarian cancer reveals global patterns and targeted DNA amplification. PLoS One 2013; 8: e80306.

27) Yong W, Yu D, Jun Z, Yachen D, Weiwei W, Midie X, Xingzhu J, Xiaohua W. Long noncoding RNA NEAT1, regulated by LIN28B, promotes cell proliferation and migration through sponging miR-506 in high-grade serous ovarian cancer. Cell Death Dis 2018; 9: 861.

28) Li J, Huang H, Li Y, Li L, Hou W, You Z. Decreased expression of long non-coding RNA GAS5 promotes cell proliferation, migration and invasion, and indicates a poor prognosis in ovarian cancer. Oncol Rep 2016; 36: 3241-3250.

29) Zhu FF, Zheng FY, Wang HO, Zheng JJ, Zhang Q. Downregulation of lncRNA TUBA4B is associated with poor prognosis for epithelial ovarian cancer. Pathol Oncol Res 2018; 24: 419-425.

30) Peng L, Yuan XQ, Liu ZY, Li WL, Zhang CY, Zhang YQ, Pan X, Chen J, Li YH, Li GC. High lncRNA H19 expression as prognostic indicator: data mining in female cancers and polling analysis in non-female cancers. Oncotarget 2017; 8: 1655-1667.