Dys-regulation of Lnc-SNHG1 and miR-216b-5p Correlate With Chemo-resistance and Indicate Poor Prognosis of Serous Epithelial Ovarian Cancer

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Research

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Abstract

Background: Ovarian cancer is the most lethal of gynecological cancers and the 5-year survival rate is still low and chemo-resistance is the main obstacle for the treatment of ovarian cancer.

Methods and Results: In this study, the human ovarian cancer tissue microarray was applied, 63 cases of chemo-sensitive serous EOC tissues and 32 cases of chemo-resistant serous EOC tissues were selected. By applying RNA fluorescence in situ hybridization (FISH), we found expression of Lnc-SNHG1 was up-regulated while miR-216b-5p showed low expression in chemo-resistant EOC patients compared with the chemo-sensitive group, both were mainly localized in the cytoplasm of the tissue cells. Chi-square test showed high lnc-SNHG1 level was significantly correlated with tumor stage, histological grade, nodal status, metastasis and chemo-resistance except tumor size. While there was no significant association between miR-216b-5p expression and parameters including tumor stage, histological grade, nodal status, metastasis, except chemo-resistance (P=0.0001). Spearman's correlation analysis revealed significantly negative correlations between lnc-SNHG1 and miR-216b-5p (r = -0.424, P = 0.0001). Multivariate Cox regression analysis showed the expression of miR-216b-5p (P = 0.012, RR 2.137, 95 % CI 1.109–5.339) and FIGO stage (P = 0.001, RR 3.537, 95 % CI 1.72–7.276) was independent prognostic factors for the overall survival (OS) of serous EOC patients .While the FIGO stage (P = 0.003, RR2.237, 95 % CI 1.323–3.783) was the independent prognostic factor for the disease free survival (DFS) for the serous EOC patients. Kaplan- Meier curves revealed significant association of increased expression of lnc-SNHG1 with less OS and shorter DFS, while patients with low level of miR-216b-5p indicated less OS and DFS.

Conclusions: In a word, we claimed over-expression of lnc-SNHG1 and decreased expression of miR-216b-5p were correlated with chemo-resistance of serous EOC patients and indicated less OS and shorter DFS of the patients.

Introduction

Over the past 30 years, the overall 5-year survival rate for cancer patients has increased by 20% as method for early screening and therapy for cancer progressed[1]. In contrast, the survival rate of ovarian cancer patients has not changed much in recent decades, and even in developed countries, the 5-year survival rate is only 47%[2]. Worldwide 239,000 new cases of ovarian cancer occur annually (3.6% of all cancer cases), of which 152,000 cases die every year (4.3% of all cancer deaths) occur annually. Ovarian cancer is also the seventh most common cancer and the first cause of death among women worldwide among gynaecological cancer [3]. About 70% of patients with ovarian cancer have been diagnosed as stage III or stage IV, and the treatment of platinum along with paclitaxel after cytoreductive surgery followed by maintenance therapy is still the recommended standard treatment for ovarian cancer at home and abroad[4]. However, at least 70% of ovarian cancer patients eventually develop chemotherapeutic resistance. Therefore, underlining the mechanism of ovarian cancer chemo-resistance is essential.

Along with the application of high-throughput sequencing technology, the research on the structure and function of non-coding RNA has been attracting the attention. The lncRNAs, as a class of non-coding RNA transcripts, are more than 200 nucleotides in length. Due to the lack of open reading frame, lncRNAs do not have the potential to directly encode proteins, but they can regulate gene expression in cis or trans through different mechanisms [5–7]. Studies have showed the dysregulation of lncRNAs were related to tumorigenesis, development, metastasis and chemo-resistance [8–10]. What' more lncRNAs have shown potential as bio-markers in many malignant tumors [11, 12].

In the year of 2011, Salmena et al. proposed a unique regulatory mechanism between lncRNAs and messenger RNAs, namely the competitive endogenous RNA (ceRNA) hypothesis, they assumed lncRNA regulates the expression of mRNAs because it contains miRNA response element (MRE) which can competitively bind to the same miRNAs[13]. That is to say, lncRNA can reduce the expression of miRNA by sponge adsorption, thus inhibiting the negative regulation of miRNA on downstream target genes [14, 15]. The ceRNA regulatory model of competitive binding of lncRNA to miRNA has become a hotspot in many malignant tumors, including ovarian cancer. However, the study of lncRNAs in chemo-resistance of ovarian cancer needs to be further explored. As a newly reported long non-coding RNA, lnc-SNHG1 (small nucleolar RNA host gene 1), was significantly over-expressed in lung cancer cell line than that in normal lung epithelial cells, after silencing lnc-SNHG1 expression, the cell proliferation was inhibited[16]. It is also reported that the expression level of lnc-SNHG1 was significantly up-regulated in liver cancer tissues and liver cancer cell line compared with normal liver tissue and cell line, and over-expression of lnc-SNHG1 promoted the proliferation, invasion and migration of liver cancer cells through binding miR-195[17].To our knowledge, whether lnc-SNHG1 is over-expressed in ovarian cancer chemo-resistant patients has not been reported.

Micro RNAs (miRNAs) play an important role in the regulation of gene expression related to cell growth cycle, cell proliferation and apoptosis and has been a hot topic in the study of chemo-resistance in ovarian cancer. Li et al. reported that miR-142-5p enhanced the sensitivity of ovarian cancer cells to platinum by targeting and inhibiting the expression of anti-apoptotic genes[18]. Biamonte et al. claimed microRNA let-7 g could be used as a tumor suppressor in epithelial ovarian cancer and as a marker to predict the chemo-sensitivity of ovarian cancer[19]. Also miR-34c and miR-383-5p were clarified to increase the sensitivity of ovarian cancer cells to chemotherapy by inhibiting the proliferation of ovarian cancer cells[20, 21]. MiR-216b was reported in a variety of tumors except ovarian cancer in recent years. Wang[22] et al. found that the expression of miR-216b in gastric adenocarcinoma was significantly lower than that in normal tissues. However, to our knowledge, whether the low expression of miR-216b-5p is related to chemo-resistance of ovarian cancer and its mechanism has not been reported.

In this study, we focus on the dysregulation of lnc-SNHG1 and miR-216b-5p in the prediction of chemo-resistance of ovarian cancer, in addition, we are to explore the association of lnc-SNHG1 and miR-216b-5p dysregulation with clinicopathological feature including chemo-resistance, metastasis, node status, tumor diameter of serous EOC patients. Finally, we aim to search the prognostic importance of

dysregulated expression of lnc-SNHG1 or miR-216b-5p for the prediction of Overall survival (OS) and disease-free survival (DFS) for the EOC patients.

Materials And Methods

Tissues and clinical data

The human ovarian cancer tissue microarray was purchased from Shanghai Outdo Biotech (Sample NO. HOvaC154Su01, Shanghai, P.R. China). The tissue microarray including 2 cases of benign ovarian tumors and 152 cases of ovarian cancer who were followed up for 5–9 years. Among which, we selected the serous epithelial ovarian cancer tissues in this study. The patients selected were divided into two groups, namely the chemo-sensitive group and the chemo-resistant group. The grouping criteria is as following: the patients who relapsed within 6 months after chemotherapy were considered as chemo-resistant, and the patients who relapsed between 6 months and 12 months after chemotherapy were considered as partial sensitive, the patients who did not relapse more than 12 months after chemotherapy were considered as chemo-sensitive. There were 63 cases of chemo-sensitive serous EOC tissues and 32 cases of chemo-resistant serous EOC tissues. Clinical features and pathological information including age (years), FIGO stage, differentiation, tumor diameter (cm), nodal status, metastasis were summarized in Table 1. Our study was approved by the ethics committee of the First Affiliated Hospital of Xi'an Jiao tong University and (approval number: 2020-G143), and the ethics committee of Shanghai Outdo Biotech Company (approval number: YB M-05-02).

Table 1
Expression of lnc-SNHG1 in serous epithelial ovarian cancers tissues

	lnc-SNHG1					χ2	P
	Total cases	High expression(n = 37)		Low expression(n = 58)			
Age (years)	n	n	n%	n	n%	0.074	0.786
Postmenopausal(< 50)	42	17	40.48%	25	59.52%		
Premenopausal(≧ 50)	53	20	37.74%	33	62.26%		
Tumor stage (FIGO)						8.435	0.015
-	21	4	19.05%	17	80.95%		
	45	16	35.56%	29	64.44%		
IV	29	17	58.62%	12	41.38%		
Histological grade (n = 88)						6.529	0.011
H	72	34	47.22%	38	52.78%		
L	16	2	12.50%	14	87.50%		
Nodal status(n)						13.323	0.0001
Negative	60	15	25.00%	45	75.00%		
Positive	35	22	62.86%	13	37.14%		
Tumor diameter (cm)						0.343	0.842
< 10 cm	30	12	40.00%	18	60.00%		
10-20cm	58	23	39.66%	35	60.34%		
> 20 cm	7	2	28.57%	5	71.43%		
Chemotherapy						31.148	0.0001
chemo-resistance	32	25	78.13%	7	21.87%		
chemo-sensitive	63	12	19.05%	51	80.95%		
Metastasis						25.499	0.0001
Y	19	17	89.47%	2	10.53%		
N	76	20	26.32%	56	73.68%		

Rna Fluorescence In Situ Hybridization (fish)

Primer for the lnc-SNHG1 FISH probe was 5'-GCAGGAAGGGGGTGATAAAATACAGAAATG – 3', while the primer for the miR-216b-5p probe was 5'- TCACATTTGCCTGCAGAGATTT-3'. The fluorescence probe of lnc-SNHG1 was labeled with Cy3 which is red, and the fluorescence probe of miR-216b-5p was labeled with FAM which is green. One tissue microarray slice was hybridized by Cy3-probes specific for the lnc-SNHG1, the other slice was hybridized by FAM-probes specific for the miR-216b-5p. Procedure of in situ hybridization was as following: 1. Slices were dewaxing to DEPC water followed by boiling in the repair solution for 15 minutes and cooled naturally. Then protease K (20ug / ml) was dripped for digesting about 25 minutes. After washing with pure water, PBS was used to wash for 3 times. 2. Pre-hybridization: slices were incubated with pre-hybridization solution at 37 ℃ for 1hour. 3. Hybridization: poured out the pre-hybridizing solution, dropped the hybrid solution containing probe of lnc-SNHG1 or miR-216b-5p (the concentration is 6 ng / UL), and the slices were hybridized in incubator at 37 ℃ overnight. 4. Washing after hybridization: washed off the hybridization solution with 2 × SSC (sodium saline citrate) for 10 min with 1 × SSC for 5 min twice, and then with 0.5 × SSC for 10 min at room temperature. 5. Slices were incubated with DAPI (4', 6-diamidino-2-phenylindole) dye solution in dark for 8 min, then anti-fluorescence quenching sealing agent was added after washing. 6. Fluorescence microscope and photo taking: the slices were observed and the images were collected under the Nikon positron fluorescence microscope (NIKON ECLIPSE CI). The nucleus stained by DAPI was blue under the excitation of UV, and the positive expression was the fluorescence labeled by corresponding fluorescein. Fam (488) is green, Cy3 is red.

Statistics

SPSS version 19.0(IBM SPSS, Chicago, IL) was used for statistical analysis. The data were expressed as mean ± SD. Student’s t-test was used to compare quantitative variables. Chi-square test was applied to determine the association of lnc-SNHG1 or miR-216b-5p and clinicopathological variables. Multivariate logistic regression analysis was applied to determine the related factors for chemo-resistance. The Cox’s proportional hazard model was applied to identify independent prognostic factors for overall survival (OS) and disease-free survival (DFS). Spearman’s correlation analysis was used to analyze the correlation between lnc-SNHG1 and miR-216b-5p expression. The OS curve and the DFS curve was analyzed by the Kaplan–Meier test. The log-rank test was used to compare OS and DFS between chemo-sensitive and chemo-resistant groups. The OS was calculated from the dates of surgery to the date of death, and the DFS was calculated from the dates of surgery and recurrence. A P value less than 0.05 was considered statistically significant.

Results

The expression of lnc-SNHG1 and miR-216b-5p and their clinical significance in serous EOC patients

FISH analysis was applied to determine the expression and cellular location of lnc-SNHG1 and miR-216b-5p in epithelial ovarian cancer patients. Results showed that lnc-SNHG1 expression was up-regulated in chemo-resistant serous EOC patients compared with the chemo-sensitive group and it was mainly localized in the cytoplasm of the tissue cells (Fig. 1A, C). While the expression of miR-216b-5p was low in chemo-resistant tissues (Fig. 1B, D). Likewise, data showed the high expression rate of lnc-SNHG1 was significantly higher in chemo-resistant group compare with the chemo-sensitive patients (86.49% vs. 13.51%, P = 0.0001) (Fig. 2A), while the high expression rate of miR-216b-5p was remarkably lower in chemo-resistant group compare with the chemo-sensitive patients (10.81% vs. 89.19%%, P = 0.0001) (Fig. 2B).

To assess whether the dysregulation of lnc-SNHG1 or miR-216b-5p was associated with clinicopathological features of serous EOC patients, the expression level of lnc-SNHG1 was classified as low (n = 58) or high (n = 37) according to the median value of lnc-SNHG1 expression. Also, based on the median value of miR-216b-5p, patients of EOC were categorized into miR-216b-5p low expression group (n = 58) and miR-216b-5p high expression group (n = 37). We found the lnc-SNHG1 high expression showed significant correlation with tumor stage, histological grade, nodal status, metastasis, and chemo-resistance except tumor size (Table 1). While there was no significant association between miR-216b-5p expression and parameters including tumor stage, histological grade, nodal status, metastasis, but only showed significant correlation with chemo-sensitivity (P = 0.0001,Table 2).

Table 2
Expression of miR-216b-5p in serous epithelial ovarian cancers tissues

	miR-216b-5p					χ^2	*P*
	total cases	High expression(n = 37)		Low expression(n = 58)			
	n	n	n%	n	n%		
Age (years)						0.074	0.786
Postmenopausal(<50)	42	17	40.48%	25	59.52%		
Premenopausal(≧50)	53	20	37.74%	33	62.26%		
Tumor stage (FIGO)						2.708	0.258
□-□	21	8	38.10%	13	61.90%		
□	45	21	46.67%	24	53.33%		
IV	29	8	27.59%	21	72.41%		
Histological grade (n = 88)						2.216	0.137
H	72	26	36.11%	46	63.89%		
L	16	9	56.25%	7	43.75%		
Nodal status(n)						2.509	0.113
Negative	60	27	45.00%	33	55.00%		
Positive	35	10	28.57%	25	71.43%		
Tumor diameter (cm)						1.065	0.587
< 10 cm	30	11	36.67%	19	63.33%		
10-20cm	58	22	37.93%	36	62.07%		
> 20 cm	7	4	57.12%	3	42.88%		
Chemotherapy						14.194	0.0001
chemo-resistance	32	4	12.50%	28	87.50%		
chemo-sensitive	63	33	52.38%	30	47.62%		
Metastasis						0.1	0.752
Y	19	8	42.11%	11	57.89%		
N	76	29	38.16%	47	61.84%		

Further, Spearman's correlation analysis revealed significantly negative correlation between lnc-SNHG1 and miR-216b-5p (r = -0.424, P = 0.0001) (Fig. 2C).

Risk Factors Related To Chemo-resistance Of Serous Eoc Patients

By applying the univariate analysis, we found the dysregulation of lnc-SNHG1 (P < 0.0001, Table1) and miR-216b-5p P = 0.0001, Table 2) level was indicated to be independent related risk factors for chemo-resistance of serous EOC patients. Besides, the multivariate logistic regression analysis was also applied in the study. Clinical features including tumor stage, histological grade, nodal status, metastasis, tumor size along with the expression of lnc-SNHG1 and miR-216b-5p level were included in the model. Forward stepwise method was selected to analyze variables and the results showed dysregulation of lnc-SNHG1 (P < 0.0001) and miR-216b-5p (P = 0.033) along with FIGO stage (P = 0.01) were factors associated with chemo-resistance of serous EOC patients (Table 3).

Table 3
Multivariable analysis of factors associated with chemo-resistance of serous EOC

	B	S.E.	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
					Lower	Upper
Lnc-SNHG1	2.159	0.583	0.0001	8.659	2.761	27.155
Tumor stage (FIGO)	1.049	0.408	0.01	2.856	1.283	6.355
miR-216b-5p	1.482	0.693	0.033	4.4	1.131	17.122

Multivariate Cox regression model result for the OS and DFS.

Multivariate Cox regression analysis was used to assess the prognostic factors indicating the OS and DFS for the serous EOC patients. Clinical features including tumor stage, histological grade, nodal status, metastasis, tumor size along with the expression of lnc-SNHG1and miR-216b-5p level were included as variables in the analysis. It comes out that the expression of miR-216b-5p (P = 0.012, RR 2.137, 95% CI 1.109–5.339) and FIGO stage (P = 0.001, RR 3.537, 95% CI 1.72–7.276) was independent prognostic factors for the OS of serous EOC patients (Table 4).While the FIGO stage (P = 0.003, RR2.237, 95% CI 1.323–3.783) was the independent prognostic factor for the DFS for the serous EOC patients (Table 5).

Table 4
Multivariable Cox regression analyses for overall survival

	B	SE	Sig.	Exp(B)	95.0% CI for Exp(B)	
					Lower	Upper
age(year)	-0.005	0.28	0.987	0.995	0.575	1.723
Lnc-SNHG1	0.422	0.317	0.183	1.525	0.819	2.842
Nodal status	0.395	0.401	0.325	1.484	0.676	3.256
Metastasis	-0.471	0.478	0.325	0.624	0.244	1.594
Tumor stage (FIGO)	1.263	0.368	0.001	3.537	1.72	7.276
miR-216b-5p	0.759	0.304	0.012	2.137	1.178	3.875

Table 5
Multivariable Cox regression analyses for disease-free survival

	B	SE	Sig.	Exp(B)	95.0% CI for Exp(B)	
					Lower	Upper
age(year)	0.08	0.25	0.749	1.083	0.664	1.768
Lnc-SNHG1	0.389	0.269	0.148	1.475	0.871	2.498
Nodal status	0.188	0.337	0.577	1.207	0.623	2.339
Metastasis	-0.053	0.401	0.895	0.949	0.432	2.082
Tumor stage (FIGO)	0.805	0.268	0.003	2.237	1.323	3.783
miR-216b-5p	0.465	0.253	0.066	1.591	0.97	2.611

Comparison Of Os And Dfs By Kaplan–meier Analysis

Patients were followed for 5–9 years in our study. Kaplan–Meier analysis indicated that patients with high expression level of lnc-SNHG1 showed significantly less OS compared with the patients with low expression level of lnc-SNHG1 (survival interval: 40.068 ± 5.678 vs 71.329 ± 4.730, $P < 0.0001$), and the DFS time was also significantly decreased for the patients with high expression level of lnc-SNHG1 (survival interval: 27.081 ± 5.345 vs 46.238 ± 4.022, $P = 0.003$). While for the expression of miR-216b-5p, patients with low level of miR-216b-5p showed shorter overall survival time (survival interval: 51.875 ± 5.267 vs 69.373 ± 5.601, $P = 0.010$) and less time of DFS (survival interval: 33.978 ± 4.413 vs45.949 ± 4.823 P = 0 .007). Together, Kaplan–Meier analysis showed that lnc-SNHG1 over-expression was associated with a significant decrease in the mortality rate and DFS, while low expression of miR-216b-5p indicated poor OS and DFS.

Discussion

For the treatment of ovarian cancer, patients routinely accepted chemotherapy of platinum along with paclitaxel after cytoreductive surgery followed by maintenance therapy. However, chemo-resistance is still the main obstacle of the successful treatment of ovarian cancer and indicates poor prognosis of ovarian cancer patients. Recent studies have shown lncRNAs could be involved in the process of chemo-resistance of cancers[23] and dysregulation of lncRNAs in the study of chemo-resistance of ovarian cancer has also been attracted much attention. Early to 2015, it was reported that high level expression of HOTAIR could induce platinum resistance in ovarian cancer cells by DNA methylation[24]. In addition, researchers found over-expression of lncRNA-FER1L4 could increase the sensitivity of ovarian cancer cells to paclitaxel by inhibiting MAPK signaling pathways[25]. Also, lncRNA-KB-1471A8.2 was reported to be down-regulated in ovarian cancer tissues and chemo-resistant ovarian cancer cells and could act as tumor-suppressor gene by inhibiting the expression of CDK4[26].

As a newly discovered lnRNA, lnc-SNHG1 is localized at 11q12.3, which has been reported be to up-regulated in many malignant tumors. You et al reported lnc-SNHG1 was significantly highly expressed in lung cancer cell lines compared with that in normal lung epithelial cells, by silencing lnc-SNHG1 expression, the cell proliferation was inhibited[16]. Similarly, Wang et al reported lnc-SNHG1 was significantly up-regulated in the glioma tissues and associated with poor overall survival of glioma patients[27]. Interestingly, Zhang et al claimed lnc-SNHG1 was up-regulated in HCC tissues compared with that in adjacent liver tissues. They found the high lnc-SNHG1 expression level was closely related to large tumor size, poor differentiation, aggressive stage, and suggested poor prognosis of HCC patients[28]. In the year of 2019, a meta-Analysis reviewed the prognostic value of lnc-SNHG1 expression in eight solid malignant tumors and indicated expression of lnc-SNHG1 was significantly correlated with reduced overall survival (OS) (HR = 1.917; 95% CI, 1.58–2.31) ($P < 0.001$), TNM stage (OR = 3.99; 95% CI, 2.48–6.43) and lymph node metastasis (OR = 3.12; 95% CI, 1.95–4.98). While there were no significant correlation between lnc-SNHG1 expression and patient gender, tumor sub-type, or tumor size[29].

To our knowledge, we claimed for the first time that lnc-SNHG1 was up-regulated in chemo-resistant EOC patients compared with the chemo-sensitive group, besides we found lnc-SNHG1 was mainly localized in the cytoplasm of the tissue cells. In addition, we found high expression of lnc-SNHG1 significantly correlated with tumor stage, histological grade, nodal status, metastasis, and chemo-resistance except tumor size. By applying the univariate analysis, we found the dysregulation of lnc-SNHG1was indicated to be independent chemo-resistant related risk factor ($P < 0.0001$), and multivariate logistic regression analysis showed tumor stage ($P < 0.0001$), expression of lnc-SNHG1 ($P = 0.01$) level were independent risk factors associated with chemo-resistance of serous EOC patients (Table 4). Kaplan–Meier analysis indicated that patients with high expression level of lnc-SNHG1 showed significantly less OS compared with the patients with low expression level of lnc-SNHG1(survival interval: 40.068 ± 5.678 vs 71.329 ± 4.730, $P < 0.0001$), and the DFS was also significantly decreased for the patients with high expression level of lnc-SNHG1(survival interval: 27.081 ± 5.345 vs 46.238 ± 4.022, $P = 0.003$). Similar to our result, Xiong et al found in their study that lnc-SNHG1 was up-regulated in breast cancer tumors, and high

expression level of lnc-SNHG1 correlated with advanced clinical stage in breast cancer tissues significantly[30]. Xu et al found in their study that lnc-SNHG1 was over-expressed in human colorectal cancer tissues, and high lnc-SNHG1 indicated poor survival of colorectal cancer patients[31]. More recently, Zhang et al. reported lnc-SNHG1 enhanced the tumorigenesis of meningioma cells through Wnt signaling pathway by sponging miR-556-5p and thus negatively regulating the expression of miR-556-5p[32].

MiR-216b has recently been reported as a tumor suppressor miRNA in multiple tumors except ovarian cancer. Research by Deng et al. reported that miR-216b expression was low in nasopharyngeal carcinoma cell lines and tissues, over expression of miR-216b could inhibit cell proliferation and invasion by targeting KRAS[33]. Xu et al in the year of 2016 reported miR-216b negatively regulated c-Jun and thus promoted cell apoptosis of endoplasmic reticulum stress[34]. Liu et al in their study reported miR-216b was significantly lower in HCC tissues than normal tissues[35]. Exogenously over-expression of miR-216b reduced its target gene expression and thus inhibited hepatoma cell proliferation, migration and invasion. Wang [22] et al. found that expression of miR-216b was significantly down-regulated in gastric adenocarcinoma tissues than in non-cancer tissues, and exogenously over-expression of miR-216b could inhibit gastric cancer cell proliferation by negatively regulating its target gene HDAC8. However, whether the low expression of miR-216b-5p related to the chemo-resistance of ovarian cancer has not been reported at home and abroad so far. By applying starBase v2.0 [36], we found lnc-SNHG1 could directly target miR-216b-5p(S Fig. 1). In order to search the expression level of miR-216b-5p and further find the correlation between lnc-SNHG1 and miR-216b in ovarian cancer patients, we also tested the expression and location of miR-216b-5p.

Our data showed the expression of miR-216b-5p was remarkably lower in chemo-resistant group compare with the chemo-sensitive patients (high expression level rate: 10.81% vs. 89.19%%, P = 0.0001) and it was mainly localized in the cytoplasm of the tissue cells. We found there was no significant association between miR-216b-5p expression and parameters including tumor stage, histological grade, nodal status ,metastasis, however low expression level of miR-216b-5p was significantly correlated with chemo-resistance .The univariate analysis showed that dysregulation of miR-216b-5p indicated to be independent risk factors for chemo-resistance (P < 0.0001) (Table 2). Besides, the multivariate logistic regression analysis was also applied and the results showed miR-216b-5p (P = 0.033) level were independent risk factors associated with chemo- resistance of serous EOC patients (Table 3). Multivariate Cox regression analysis indicated that dysregulation of miR-216b-5p (P = 0.012, RR 2.137, 95% CI 1.109–5.339) and FIGO stage (P = 0.001, RR 3.537, 95% CI 1.72–7.276) was independent prognostic factors for the OS of serous EOC patients (Table 4).While the FIGO stage (P = 0.003, RR2.237, 95% CI 1.323–3.783) was the independent prognostic factor for the DFS for the serous EOC patients (Table 5). Kaplan–Meier analysis indicated that patients with low level of miR-216b-5p showed shorter OS (survival interval: 51.875 ± 5.267 vs 69.373 ± 5.601, P = 0.010) and less DFS (survival interval: 33.978 ± 4.413 vs45.949 ± 4.823 P = 0 .007). By using Spearman's correlation analysis we found significantly negative correlations between lnc-SNHG1 and miR-216b-5p (r = -0.424, P = 0.0001) .Interestingly, similar to our study, in the year of 2018, You et al.[37] in their study showed decreased expression of miR-216b-5p was significantly

associated with large tumor size and advanced TNM stage. What's more, low expression level of miR-216b-5p was associated with overall survival by applying both Kaplan-Meier and multivariate survival analysis. To our knowledge, there was no report about the correlation between lnc-SNHG1 and miR-216b in ovarian cancer patients. In our future research, we are to find whether lnc-SNHG1 will sponge miR-216b-5p and thus affect the chemo-resistance of ovarian cancer cells.

In Conclusion, this study is an important one that showed dysregulation of lnc-SNHG1 and miR-216b-5p in chemo-resistance of serous EOC patients. Over-expression of lnc-SNHG1 and low level of miR-216b-5p closely related to chemo-resistant feature and could both indicate the poor OS and DFS of the serous EOC patients. By using Spearman's correlation analysis we found significantly negative correlations between lnc-SNHG1 and miR-216b-5p. In our feature study, we are to find whether there is regulation of lnc-SNHG1 binding to miR-216b-5p thus affecting the biological function concerning the chemo-resistance of ovarian cancer.

Declarations

Ethics approval and consent to participate: Our study was approved by the ethics committee of the First Affiliated Hospital of Xi'an Jiao tong University and (approval number: 2020-G143), and the ethics committee of Shanghai Outdo Biotech Company (approval number: YB M-05-02).

Consent for publication: Not applicable.

Availability of data and materials: All data generated or analysed during this study are included in this published article

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Author's roles: T.S. was involved in study design, execution and analysis, and final approval of the manuscript. M.P. was involved in execution and acquisition of the data, manuscript drafting. X.Z. contributed to execution and the analysis of the data, manuscript drafting and final approval of the manuscript. The final manuscript and order of authorship have been approved by all authors.

Conflicts of interest: The authors declare no conflict of interest.

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Figures

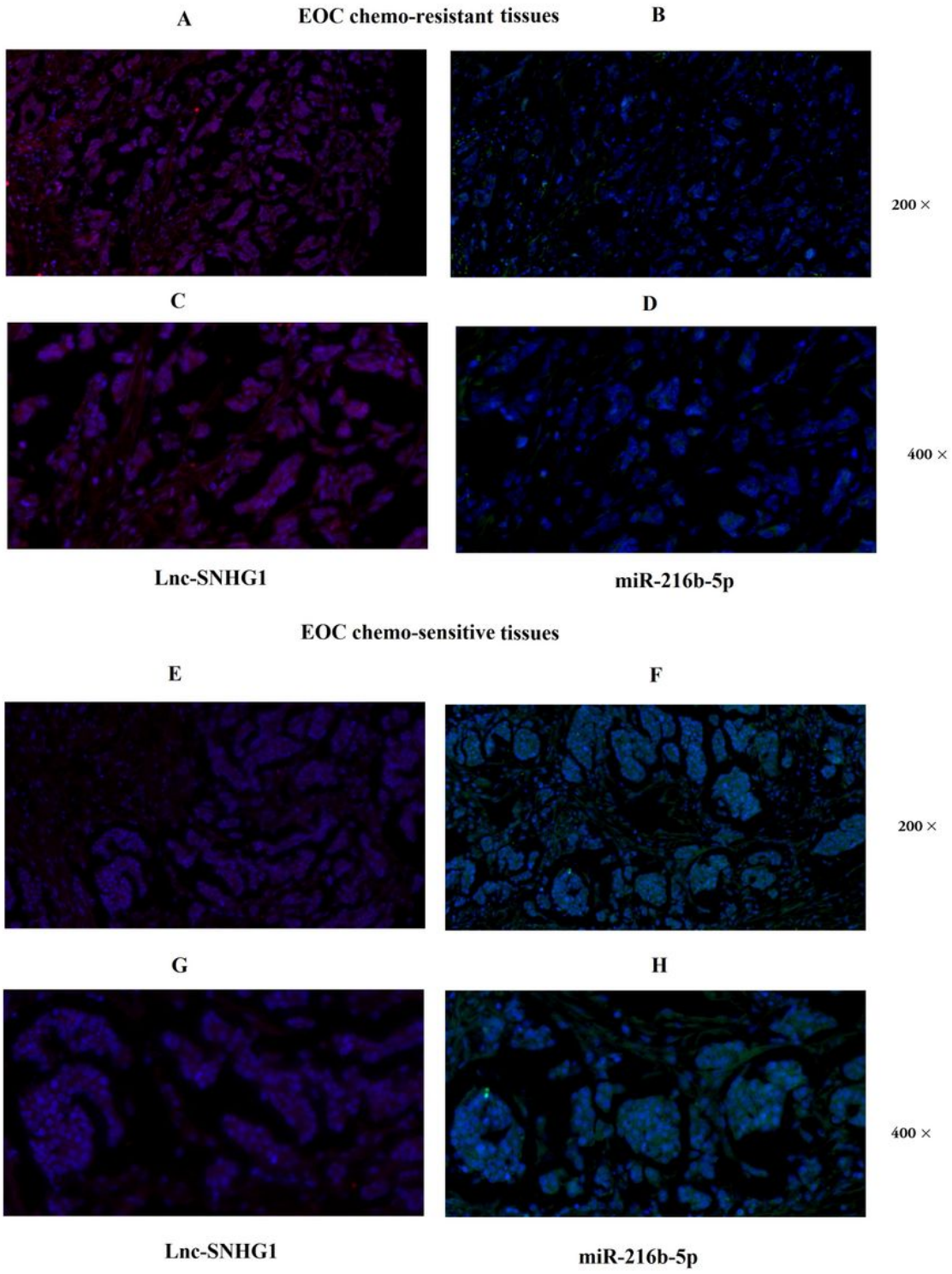
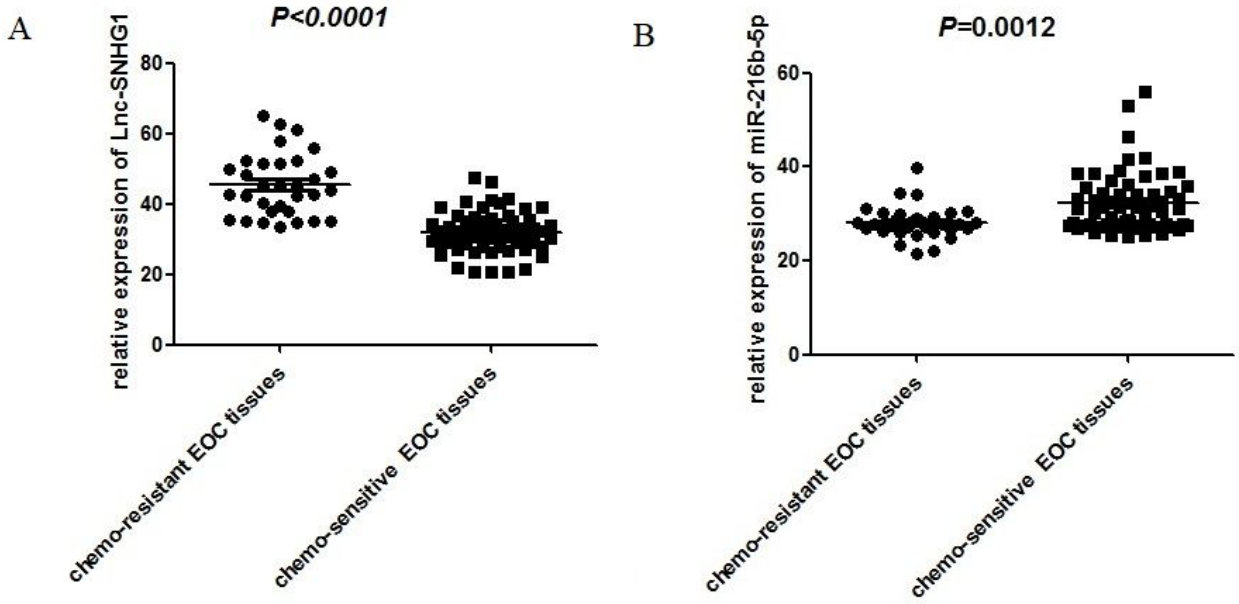


Figure 1

Expression and cellular localization of lnc-SNHG1 and miR-216b-5p were tested by Fluorescence in situ hybridization(FISH) analysis. (A,C) shows the expression of lnc-SNHG1 in chemo-resistant tissue. A,200×, B,400×. (B,D) shows the expression of miR-216b-5p in chemo-resistant tissue. B, 200×, D,400×. (E,G) shows the expression of lnc-SNHG1 in chemo-sensitive tissues. E,200×,G,400×. (F,H) shows the expression of miR-216b-5p in chemo-sensitive tissues F,200×,H,400×.



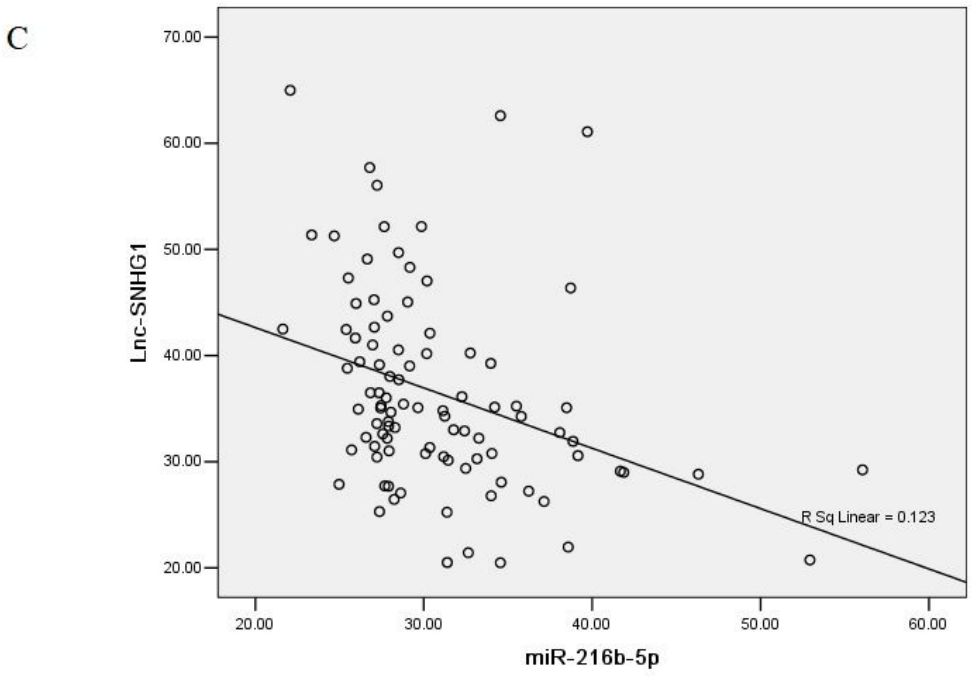


Figure 2

FISH analysis was applied for the expression of lnc-SNHG1 and miR-216b-5p in serous chemo-resistant and chemo-sensitive tissues. (A).Lnc-SNHG1 was significantly up-regulated in chemo-resistant serous EOC tissues, miR-216b-5p showed low expression in chemo-resistant serous EOC specimens. compared with that in chemo-sensitive tissues. (B). Spearman's correlation analysis was applied and showed correlations between lnc-SNHG1 and miR-216b-5p in serous EOC patients,the correlation coefficient 'r' was calculated.

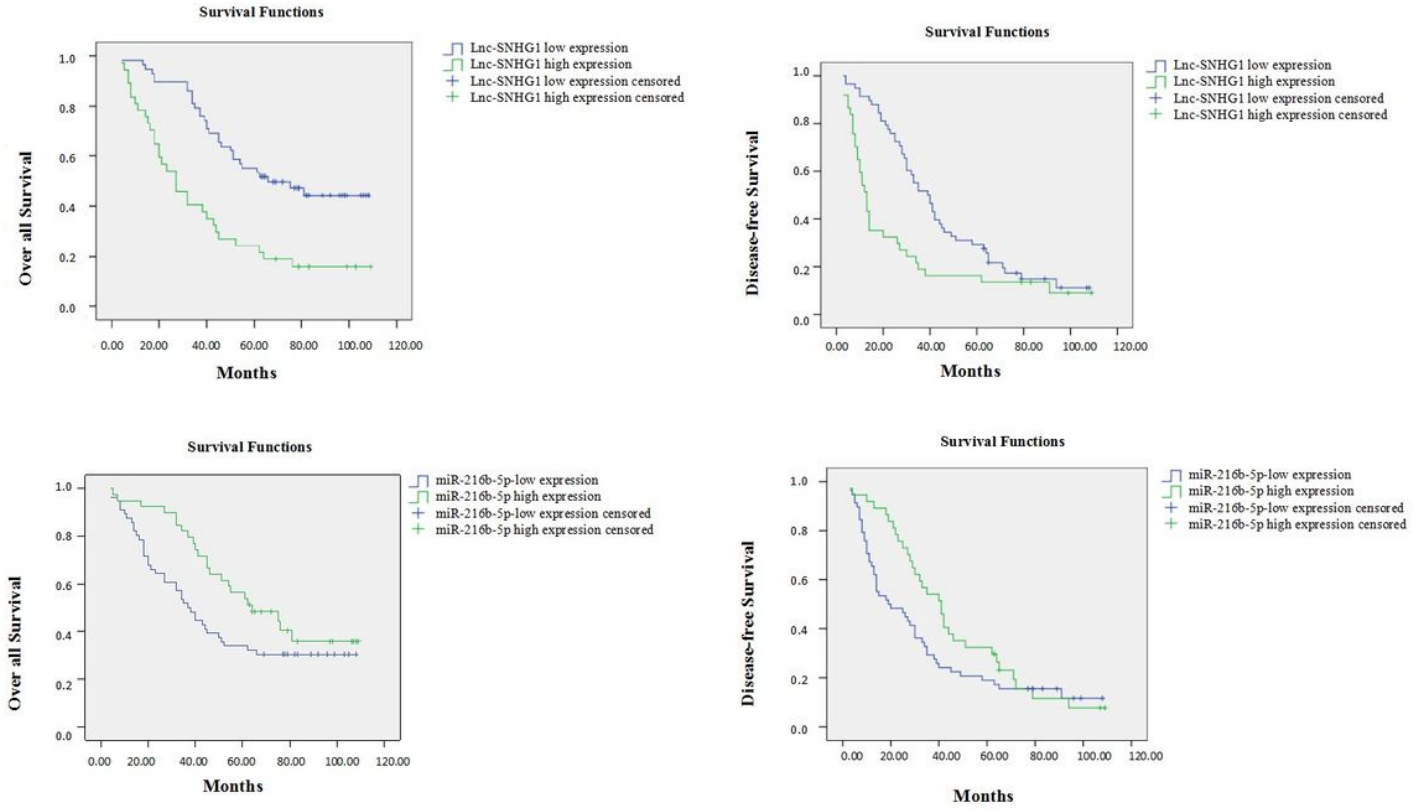


Figure 3

Kaplan-Meier survival analysis was applied for the correlation of lnc-SNHG1 and miR-216b-5p expression with OS and DFS of serous EOC patients. (A) Patients with high lnc-SNHG1 expression showed significantly shorter OS (P = 0.019, survival interval: 59.045±4.373 vs 41.714 ±4.3 months); and shorter DFS (P = 0.019, survival interval: 59.045±4.373 vs 41.714 ±4.3 months) than those with low lnc-SNHG1 expression. (B) Patients with low miR-216b-5p expression showed significantly shorter OS (P = 0.019, survival interval: 59.045±4.373 vs 41.714 ±4.3 months); and shorter DFS (P = 0.019, survival interval: 59.045±4.373 vs 41.714 ±4.3 months) than those with high miR-216b-5p expression.