



Role of lncRNAs in ovarian cancer: defining new biomarkers for therapeutic purposes

Manish K. Tripathi, Kyle Doxtater, Fatemeh Keramatnia, Chidi Zacheaus, Murali M. Yallapu, Meena Jaggi and Subhash C. Chauhan



Department of Pharmaceutical Sciences and Center for Cancer Research, University of Tennessee Health Science Center, Memphis, TN, USA

Long noncoding RNAs (lncRNAs) are a class of noncoding RNA, involved in regulation of diverse physiological and pathological processes. Ovarian cancer is the leading cause of death among all gynecological malignancies in the world and its underlying mechanism is still unclear. lncRNAs exhibit multiple biological functions in various stages of ovarian cancer development. We will discuss and summarize the new and important lncRNAs and their involvement in disease, which might represent promising therapeutic targets. Therapeutic intervention based on silencing or functional inhibition of target lncRNAs will be beneficial for ovarian cancer patients.

Introduction

Humans were supposed to have many more genes than less complex organisms. However, the number is not much different than the estimated number of genes (20,000) in roundworm *Caenorhabditis elegans* or mice, indicating complexity does not correlate with the number of protein-coding genes. However, the complexity definitely correlates with the percentage of non-protein-coding sequences. Ninety-seven percent of the human genome is non-protein-coding, consisting of introns, regulatory sequences and noncoding RNAs [1]. A type of noncoding RNA, long noncoding RNA (lncRNA) is longer than 200 nucleotide transcripts in length, has a variety of biological functions and is closely associated with tumor development. lncRNAs provide a novel way of regulating the gene expression and function at all levels of DNA, RNA or proteins. lncRNAs are at the epicenter of understanding how the vast sequence in the genome regulates different pathways including cancer. They hold an enormous potential to understand the participation of the noncoding genome in different processes and can help to bridge the vast gap that exists between cancer drug discovery and treatment. There has been an exponential rise in the number of publications during the past few years related to the role of lncRNAs in cancer biology

(Fig. 1). Not many lncRNAs have been functionally characterized, many of the lncRNAs have been identified by revisiting the array datasets on the publicly available resources [2,3], making it necessary to understand the mechanisms and biology of this new class of regulators, which is now at the center of various physiological and pathological processes. This information will be very useful in developing biomarker-driven cancer therapeutics.

Ovarian cancer

Ovarian cancer (OvCa) is the leading cause of death among gynecological malignancies in the world, and recurrent OvCa is almost always incurable [4]. Its underlying mechanism is still unclear. Several genetic and environmental factors have been shown to be implicated in the development of this type of cancer. The International Federation of Gynecology and Obstetrics (FIGO) Committee on Gynecologic Oncology has provided a staging system for OvCa [5]. The lack of specific signs and symptoms and the deficiency in screening programs have resulted in the late-stage diagnosis of OvCa, which in turn leads to the poor survival of these patients. The overall 5-year survival rate is low for the advanced stages OvCa owing to late diagnosis (because of its asymptomatic nature) and resistance to conventional carboplatin plus Taxol chemotherapy or relapse.

Better understanding of OvCa biology is needed to improve its management and early diagnosis. Such defects have necessitated

Corresponding authors: Tripathi, M.K. (mtripath@uthsc.edu), Chauhan, S.C. (schauha1@uthsc.edu)

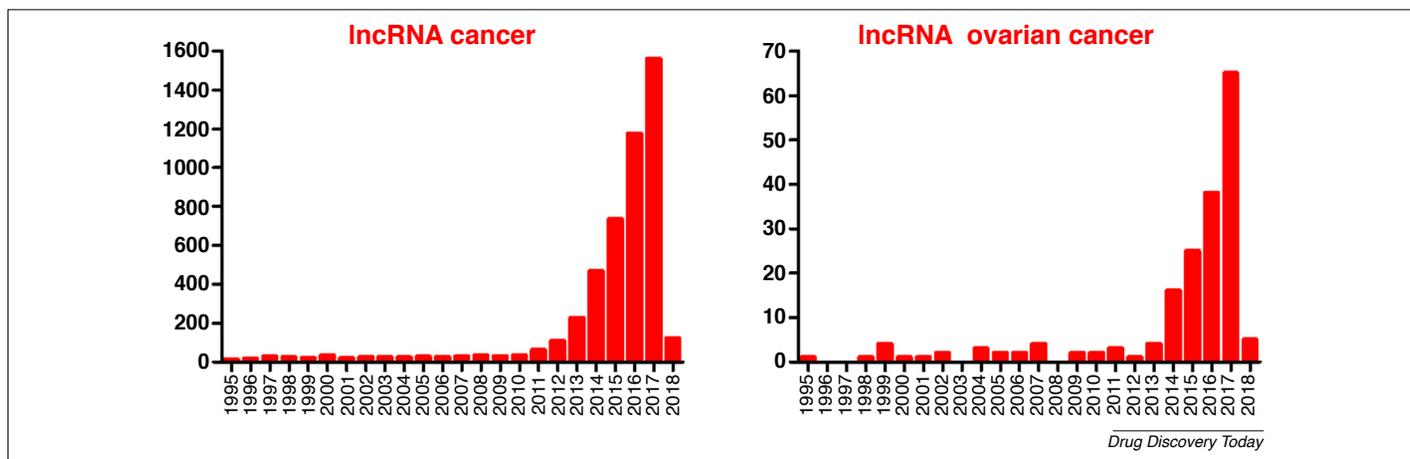


FIGURE 1 Increasing number of articles about lncRNA and cancer, and lncRNA specific to ovarian cancer. PubMed was searched with keywords ‘lncRNA Cancer’ or ‘lncRNA Ovarian Cancer’ (<https://www.ncbi.nlm.nih.gov/pubmed>).

the implementation of experimental approaches and clinical studies to discover and assess biomarkers associated with early-stage disease [6]. Compared with research on microRNAs, research on lncRNAs is still in its infancy. Studies in recent years have demonstrated that lncRNAs exhibit multiple biological functions in various stages of OvCa development. lncRNAs are closely involved in the pathogenesis of OvCa. The expression of lncRNAs indicates the early diagnosis, prognosis and response to chemotherapy of OvCa. Further research efforts are needed before fully identifying, characterizing and elucidating the actual functions of lncRNAs in OvCa at the molecular level and putting them into clinical practice [7]. Reports on lncRNAs associated with OvCa are very disjointed, which makes it necessary for an effort to study and develop a signature based on lncRNAs associated with OvCa recurrence to facilitate better OvCa therapy [4].

lncRNA-based therapeutics, biogenesis and mode of action

lncRNAs are important for the activation or repression of genes relevant to a variety of disorders. They work either as tumor suppressors or oncogenes or both. The lncRNAs are very difficult to identify from a suitable classification method. No unified

mechanism of classification of lncRNAs exists. The most common relatively convenient way to classify lncRNAs depends on the genomic context, which is the position in the chromosome where the lncRNA is transcribed. The five major known classes are: natural antisense transcript, pseudogenes, large intergenic non-coding RNA, long intronic ncRNAs and other uncharacterized and divergent transcripts [8]. Figure 2 depicts the lncRNAs on the basis of their genomic context or biogenesis [9]. On the basis of targeting mechanisms, lncRNAs can be classified as: (i) Signal, – show cell-type-specific expression and respond to diverse stimuli; (ii) Decoy, – bind and titrate away a protein target, but do not exert any additional functions; (iii) Guide, – bind proteins and then direct the localization of ribonucleoprotein complex to specific targets; (iv) Scaffold, – serve as central platforms to bring together multiple proteins to form ribonucleoprotein complexes; and (v) Enhancer, – loops chromosomes to bring the effective proteins together [8,10,11]. Figure 3 describes different modes of action of lncRNAs except signal mechanism. Based on different modes of action, strategies based on silencing (small interfering RNA (siRNA), antisense oligos, ribozymes, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), Zinc Finger (ZNFs) and Transcription Activator-Like Effector Nuclease (TALENs) and

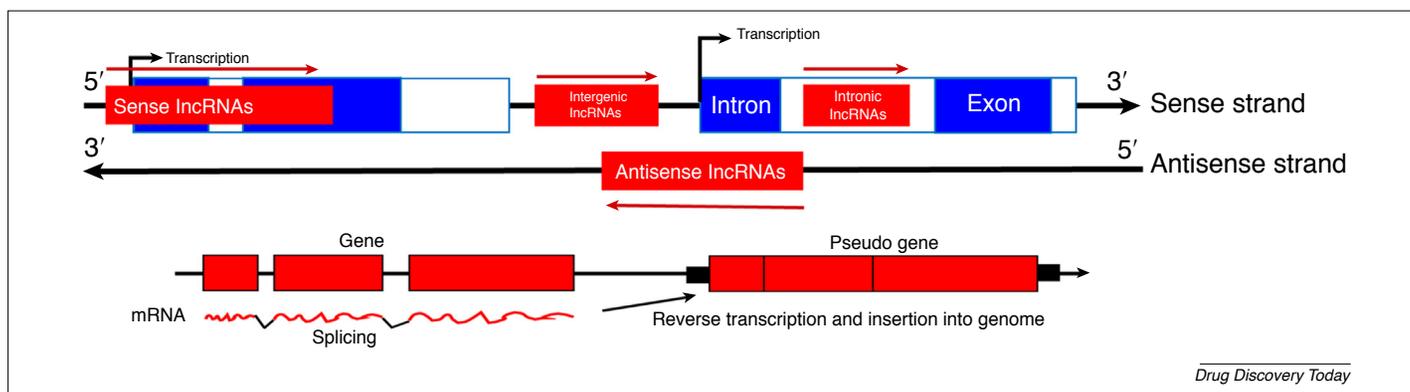


FIGURE 2 Five classes of lncRNAs based on biogenesis. Sense lncRNAs overlap with coding genes on the same strand. Antisense lncRNAs overlap with protein-coding genes on the opposite strand. Intronic lncRNAs occur completely within an intron. Intergenic lncRNAs occur between two genes. Pseudogene lncRNAs are the product of reverse transcribed mRNA and inserted into genome. Modified and adapted from reference [9], which is an open access article.

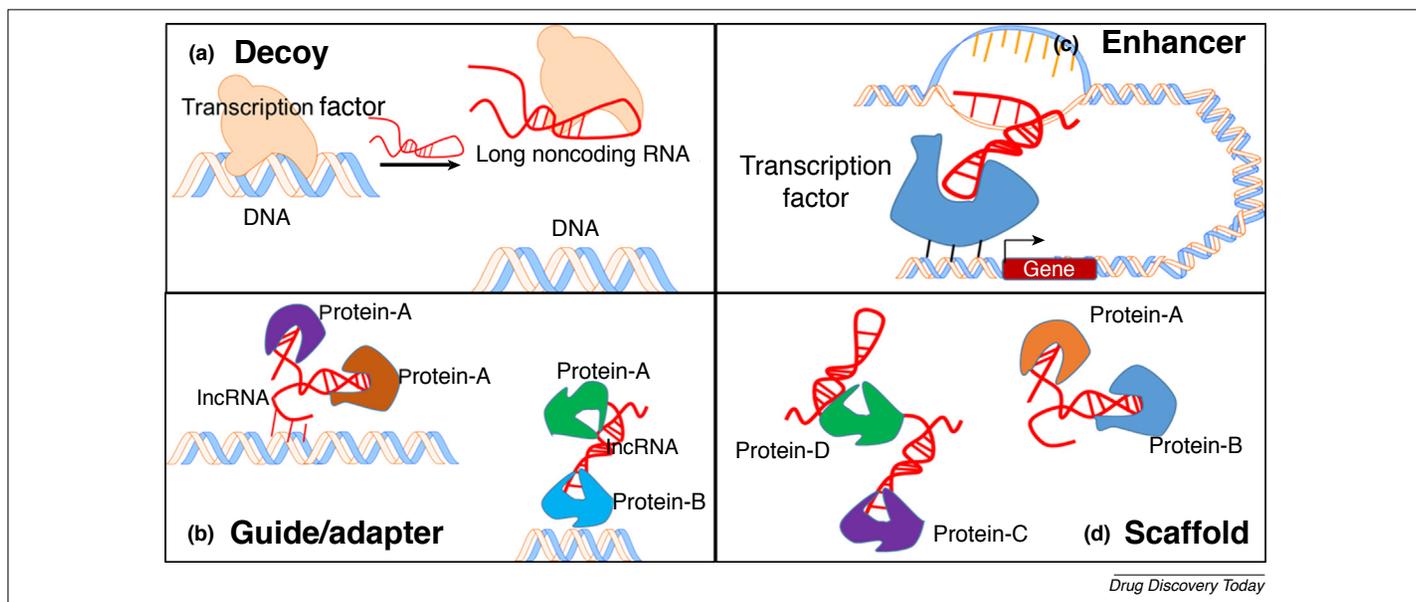


FIGURE 3

Targeting mechanisms of lncRNA. (a) Decoy, compete out transcription factors and other DNA binding proteins; (b) Guide/Adapter, modulate interactions with chromatin modifiers via RNA–DNA interactions or RNA interaction with a DNA-binding protein; (c) Enhancer, lncRNA stabilizes chromosomal looping in an enhancer-like model. (d) Scaffolds, facilitates protein–protein interaction of two or more proteins into a complex or spatial proximity. Modified and adapted considering concept from reference [11].

functional inhibition (small molecules, nanobodies, aptamers and RNA decoys) can be utilized for lncRNA therapeutics [12].

lncRNAs identified in ovarian cancer

lncRNAs are a new aspect to understanding OvCa – to diagnose and design new therapeutic approaches. Because the information on lncRNAs and OvCa is very fragmented, we made a comprehensive search for available information on lncRNAs related to OvCa on different databases such as NIH, ENCODE, Lnc2cancer, lncRNA disease database (cuilab), lncRNAdb and NONCODE. Transcription factor binding sites were analyzed using QIAGEN (GeneCards.org) and the ChIPseq database on the UCSC genome browser (ENCODE). In this review, we will summarize lncRNAs and their involvement in OvCa. The potential translation of this knowledge to diagnose and design therapeutic approaches for OvCa therapy will be very encouraging with the advent of cutting-edge translational research. Table 1 summarizes all the lncRNAs reported to date in relation to OvCa [4,13–57]. Five major lncRNAs responsible for OvCa etiology have been discussed in detail.

H19

H19 is a metastatic lncRNA that induces cell cycle arrest and apoptosis through certain cell-cycle-related and apoptosis-related proteins, it was identified by profiling 70 pairs of OvCa tissue [20,56]. H19 also showed relation to acquired drug resistance toward cisplatin chemotherapy; and high-grade serous ovarian cancer (HGSC) tissues showed strong correlation with cancer recurrence with H19 expression levels [54,58,59]. H19 RNA was detected in 90% of patients with ovarian cancer ascites fluid (OCAF) so, in an effort to develop targeted therapy for OvCa, the therapeutic potential of the toxin vector DTA-H19 was tested in ovarian carcinoma cell lines and in a heterotopic animal model for OvCa. DTA-H19 is a plasmid expressing diphtheria toxin under the control of H19 regulatory sequences [39].

HOX antisense intergenic RNA (HOTAIR)

Abnormal expression of HOTAIR and its common variants are associated with risk of epithelial ovarian cancer (EOC). HOTAIR was significantly high in 44 OvCa tissues as compared with 14 normal ovary tissues [28]. Elevated HOTAIR expression leads to chemoresistance by activating the Wnt/ β -catenin pathway in human OvCa [35], also overexpression of HOTAIR predicts poor patient prognosis and promotes tumor metastasis in epithelial OvCa. HOTAIR levels were highly positively correlated with the FIGO stage, the histological grade of the tumor, lymph node metastasis and reduced overall survival (OS) and disease-free survival (DFS). The pro-metastatic effects of HOTAIR were mediated by the regulation of the expression of a number of genes involved in cell metastasis and EMT, including matrix metalloproteinase (MMP)3, MMP9, E-cadherin, vimentin and Snail [43,58,59]. Upregulation of HOTAIR induced platinum resistance in OvCa, and increased HOTAIR levels were observed in recurrent platinum-resistant ovarian tumors vs primary ovarian tumors. The nuclear factor (NF)- κ B-HOTAIR axis links DNA damage response, chemoresistance and cellular senescence in OvCa [40]. In a clinical study of serous ovarian cancer (SOC), HOTAIR overexpression was correlated with an advanced FIGO stage and a high histological grade [44].

Metastasis-specific lung adenocarcinoma transcript (MALAT1)

MALAT1 is a potential biomarker for tumor growth and metastasis, as well as a promising therapeutic target in OvCa. Differential RNA analysis of SKOV3 (parental) and SKOV3.ip1 (metastatic) cells identified it as a metastatic specific lncRNA, and overexpressed MALAT1 in SKOV3 cells promoted cell proliferation, migration and invasion [20,55]. MALAT1 expression was associated with the FIGO stage. Knockdown of MALAT1 expression in OVCAR3 cells inhibited cell proliferation, migration and invasion, leading to G0/G1 cell cycle arrest and apoptosis. Transforming growth factor (TGF) β 1 has been shown to induce MALAT1 expression and

TABLE 1

LncRNAs having regulatory roles in ovarian cancer.

lncRNA	Name	Alias	Chromosome location: (strand)	NCBI/ENSMBL	Expression pattern	Functional/clinical info
AB073614 [13]	NA	NA	Chr3:148891375-148893279(−)	NA	Up	Tumorigenesis, poor prognosis (OS)
AL132709.8 [4]	NA	NA	Chr14q32.31	NA		Recurrence marker
AC104699.1.1 [14]	NA	NA	NA	NA		Predictive survival
ANRIL [15]	Antisense noncoding RNA in the INK4 locus (ANRIL)	CDKN2B-AS1; p15AS; CDKN2BAS; CDKN2B-AS; NCRNA00089	Chr9:21994778-22121097(+)	NR_003529	Up	Metastasis, poor prognosis-survival
ASAP1-IT1 [16]	ASAP1 intronic transcript 1	ASAP1-IT; ASAP1IT; DDEF1IT1; HSPC054; NCRNA00050	Chr8:130295355-130296533(−)	NR_002765		Fav OS
BACE1-AS [17]	β-site APP cleaving enzyme-1 antisense strand	BACE1-AS1; NCRNA00177	Chr11:117291346-117292170(+)	NR_037803	Down	Tumor suppressor
BCYRN1 [22]	Brain cytoplasmic RNA1	BC200; BC200a; LINC00004; NCRNA00004	ChrX:70430,035-70948962(−)	NR_001568; AF20057	Up and down	Tumor suppressor
CCAT1 [20]	Colon cancer associated transcript 1	CARLo-5	Chr8:127207866-127219088(??)	NR_108049	Down	Metastatic
CCAT2 [19]	Colon cancer associated transcript 2	NCCP1; LINC00873	Chr8:127400399-127402150(+)	NR_109834	Up	Short OS and DFS, positive with tumor grade and distant metastasis
CRNDE [21]	NA	NA	Chr16:54845189-5492918912(−)	ENST00000613942	High	Oncogenic, poor prognosis
FALEC [18]	Focally amplified long noncoding RNA in epithelial cancer	FAL1	Chr1:150488233-150490508(+)	NR_051960	Up	
FAM215A [16]	Family with sequence similarity 215 member A	APR-2; C17orf88; LINC00530	Chr17:43917208-43917987(+)	NR_026770	Up	Fav OS
GACAT3 [23]	Gastric cancer associated transcript 3	LINC01458; lncRNA-AC130710	Chr2:16050427-16085689(+)	NR_126559		Nonequivalent outcome
GAS5 [54,56]	Growth arrest specific 5	GAS5; SNHG2; NCRNA00030	Chr1:173863900-173868882(−)	NR_002578	Down	
H19 [20,39,54,56]	H19, imprinted maternally expressed transcript	ASM; ASM1; BWS; D11S813E; LINC00008; NCRNA00008; WT2	Chr11:1995163-2001470(−)	NR_131223	Up	Tumorigenesis, recurrence marker, metastasis
HOST2 [29]	Human ovarian-cancer-specific transcript 2	CERNA2	Chr10:84167228-84172076(−)	NR_134505	Up or down	Risk factor
HOTAIR [28,35,40,43,44]	HOX transcript antisense RNA	HOXAS; HOXC-AS4; HOXC11-AS1; NCRNA00072	Chr12:53962308-53974956(−)	NR_003716	Up/differential	Prognostic metastatic marker
HOXA11-AS [46]	HOXA11 antisense RNA	HOXA-AS51; HOXA11AS; HOXA11S; NCRNA00076; HOXA11-AS	Chr7:27185408-27189293(+)	NR_002795	Down	Oncogenic
HOTAIRM1 [4]	HOXA transcript antisense RNA, myeloid-specific 1	HOXA1-AS1; HOXA-AS1; NCRNA00179	Chr7:27135713-27139877(−)	NC_000007.14		Recurrence marker
LINC00472 [16]	Long intergenic non-protein-coding RNA 472	C6orf155	Chr6:71407864-71420745(−)	NR_121612		Fav OS
LINC00515 [54]	NA	LINC00515; PRED21; C21orf71	Chr21:25582770-25583326(−)	ENSG00000260583	Down	
LINC00961 [54]	NA	NA	Chr9: 35909483-35937153(+)	ENSG00000235387	Up	

TABLE 1 (Continued)

lncRNA	Name	Alias	Chromosome location: (strand)	NCBI/ENSEMBL	Expression pattern	Functional/clinical info
linc-CARS2-2 [54]	Cysteinyl-tRNA synthetase 2, mitochondrial	FLJ12118	Chr13:110641584-10644681(−)	ENST00000542774	Up	
lin-RECK-3 [54]	reversion inducing cysteine rich protein with kazal motifs	ST15, hRECK	Chr9:36036924-36086181(+)	ENST00000475774	Up	
linc-TNFRSF19-1 [54]	TNF receptor superfamily member 19	TAJ-alpha, TROY, TAJ, TRADE	Chr13:23579359-23594411(+)	ENST00000464735	Down	
LOC100128881 [20]	VPS9D1 antisense RNA1	LOC100128881; VPS9D1-AS1	Chr16:89711856-89718165(+)	NR_036480	Down	Metastasis
LOC100190986 [4]	Uncharacterized	NA	Chr16:21432003-21434455(−)	NA		Recurrence marker
LOC100292680 [20]	Long intergenic non-protein-coding RNA 942	LOC100292680; LINC00942	Chr12:1500525-1507318(−)	NR_028415	Down	
LSINCT5 [49]	Long stress-induced noncoding transcript 5	NA	Chr5:2712591-2715237(−)	NR_145480	Up	
LUCAT1 [54]	Lung cancer associated transcript 1	LUCAT1; SCAL1	Chr5:91303029-91314402(−)	NR_103548	Up	
MALAT1 [4,20,27,33,41,55,57]	Metastasis-associated lung adenocarcinoma transcript 1	HCN; LINC00047; NCRNA00047; NEAT2; PRO2853	Chr11:65497679-65504494(+)	NR_002819	Up	Recurrence marker
MEG3 [48,51,53]	Maternally expressed 3	FP504; GTL2; LINC00023; NCRNA00023; PRO0518; PRO2160; onco-lncRNA-83; prebp1	Chr14:100826108-100861026(+)	NR_046472		
MIR22HG [36]	NA	C17orf91	Chr17:1711504-1716272(−)	NR_028502		Oncogene, prognostic marker
MXN1-AS1 [20]	MXN1 antisense RNA 1	CCAT5; MXN1-AS1; LOC645249	Chr7:157010805-157016426??	NR_038835	Down	
NEAT1 [8,26]	Nuclear paraspeckle assembly transcript 1	LINC00084; NCRNA00084; TncRNA; VINC	Chr11:65422798-65445540(+)	NR_131012		Clinical biomarker
NRCP [47]	NA	NA	NA	NA		
OVAAL [24]	Ovarian adenocarcinoma-amplified long noncoding RNA	LINC01131; OVAL	Chr1:180558974-180566518(+)	NR_125716		
P15INK4B [15]	Cyclin-dependent kinase inhibitor 2B	P15; MTS2; TP15; CDK4I; INK4B; p15INK4b; CDKN2B	Chr9:22002903-22009363(−)	ENSG00000147883	Down	
PTPRD-AS1 [23]	PTPRD antisense RNA1		Chr9:8858018-8861727(+)	NR_121600		Nonequivalent outcome
PVT1 [31,37,38]	Plasmacytoma variant translocation 1 oncogene	LINC00079; MYC; NCRNA00079; onco-lncRNA-10; PVT1; LINC00079; NCRNA00084	Chr8:127794533-128101253(+)	NR_003367	Up	Oncogene
RP1-223E5.4 [23]	NA	AL441883.1	Chr6:13614111-13615155(−)	ENST00000566170NOTFOUND		Nonequivalent outcome
RP4-799P18.3 [23]	NA	AL122008.4	Chr1:234268583-234272500(−)	ENST00000446433		Nonequivalent outcome
RP11-254122.1 [23]	NA	MIR583HG	Chr5:96050115-96215519(+)	ENST00000507997		Nonequivalent outcome
RP11-284N8.3.1 [14]	NA	NA	NA	NA		Predictive survival
RP11-307C12.11 [23]	NA	DCST1-AS1	Chr1:155045191-155046118(−)	ENST00000452962		Nonequivalent outcome
RP11-57P19.1 [23]	NA	AC009432.1	Chr15:94600014-94600821(+)	ENST00000560391		Nonequivalent outcome
RP11-80H5.7 [23]	NA	AL157400.4	Chr10:89694295-89697928(−)	ENST00000455699		Nonequivalent outcome
RUNX1-IT1 [4]	NA	NA	Chr21q22.12	NA		Recurrence marker
TC0100223 [45]	NA	NA	NA	NA	Down	
TC0101441 [42,45]	NA	NA	Chr1:202377159-202378011	NA	Down	Prognostic factor OS
TC010686 [45]	NA	NA	Chr1:244341256-244343791	NA	Up	

TABLE 1 (Continued)

lncRNA	Name	Alias	Chromosome location: (strand)	NCBI/ENSMBL	Expression pattern	Functional/clinical info
TC1500845 [45] TUG1 [34]	NA Taurine upregulated 1	NA LINC00080; NCRNA00080; TI-227H	Chr15:38773376-38774597 Chr22:30969211-30979395(+)	NA NR_002323	Up	
UCA1 [20,50,52]	Urothelial cancer associated 1	CUDR; LINC00178; NCRNA00178; UCAT1; onco-lncRNA-36	Chr19:15828947-15836321(+)	NR_015379	Down	
XIST [32]	X inactive specific transcript	DXS1089; DXS399E; LINC00001; NCRNA00001; SX11; swd66	ChrX:73820651-73852753(-)	NR_001564	Down	
ZNF300P1 [30]	NA	NA	Chr5:150930645-150946289(-)	ENST00000356555		

subsequent phosphorylation of Mitogen-Activated Kinase Kinase 1 (MEK1), Mitogen-Activated Protein Kinase 3 (ERK1), Mitogen-Activated Protein Kinase P38 Alpha (p38) and Mitogen-Activated Kinase 8 (JNK1) suggested MALAT1 promoted OvCa cell proliferation, migration and invasion, and that mitogen-activated protein kinase (MAPK) pathways might be one of the regulatory mechanisms of MALAT1 [57]. MALAT1 also promotes proliferation and metastasis in EOC via the PI3/AKT pathway [33,59]. Plasma MALAT1 could act as a valuable biomarker for the diagnosis of metastasis. Plasma MALAT1 was significantly increased in the EOC/distant metastasis group compared with the EOC/NDM. Multivariate analysis indicated that overexpression of MALAT1, differentiation (poor), tumor-node-metastasis stage IV, lymph node metastasis (N3), peritoneal invasion (present) and higher serum carbohydrate antigen 125 levels were independent predictors of survival in patients with EOC. Survival analysis revealed that patients with increased MALAT1 expression had a poorer disease-free survival time [27]. MALAT1 also functions as a sponge for miR-200c and regulates miR-506 by targeting iASPP [60]. MALAT1 accumulates to high levels in the nucleus, where it has crucial roles in cancer progression and the formation of nuclear paraspeckles [61].

Plasmacytoma variant translocation (PVT1)

PVT1 and Myc contribute independently to ovarian and breast pathogenesis when overexpressed because of genomic abnormalities. PVT1-mediated inhibition of apoptosis might explain why amplification of 8q24 is associated with reduced survival duration in patients treated with agents that act through apoptotic mechanisms. Amplification of PVT1 contributes to the pathophysiology of ovarian and breast cancer [31,58,59]. Overexpression of lncRNA PVT1 in OvCa cells promotes cisplatin resistance by regulating apoptotic pathways. The level of PVT1 was significantly higher in OvCa tissues of cisplatin-resistant patients and cisplatin-resistant cells. The mRNA levels and protein expression of TGFβ1, p-Smad4 and caspase-3 were much higher in cisplatin-resistant cells transfected with siPVT1 [38]. Carboplatin and docetaxel have been developed as the first-line drug treatment for ovarian carcinoma. lncRNA PVT1 could be a central downstream target of carboplatin plus docetaxel because expression of PVT1 positively correlates with anticancer action of carboplatin plus docetaxel. p53 and tissue inhibitor of matrix metalloproteinase (TIMP)1 were mediated by lncRNA PVT1, which could explain partially the anticancer activity of lncRNA PVT1 [37].

Urothelial carcinoma associated (UCA1)

UCA1 functions as an oncogene, which promotes cancer cell proliferation, invasion and metastasis, and is responsible for drug resistance. UCA1 overexpression predicts clinical outcome of patients with OvCa receiving adjuvant chemotherapy. High UCA1 expression was an independent prognostic marker of poor outcome. UCA1 could serve as an indicator of response to chemotherapy and prognosis of OvCa and plays an important part in the progression of OvCa. Differential RNA analysis of SKOV3 (parental) and SKOV3.ip1 (metastatic) cells also identified UCA1 as a metastatic specific lncRNA [20]. UCA1 is related to serine/threonine protein kinase (SRPK)1 in cisplatin resistance in human OvCa cells. UCA1 can improve cell migration, invasion and induce

cisplatin resistance. SRPK1 and the apoptosis pathway proteins could be involved in the effect of UCA1 [50,59]. UCA1 expression has been correlated to metastasis in EOC. UCA1 was not only aberrantly upregulated in EOC tissues and cells but also correlated with status of lymph node metastasis and the FIGO stage. UCA1 was a prognostic factor for overall survival in EOC patients. UCA1 could also function as an endogenous sponge by directly binding to miR-485-5p. Depletion of UCA1 was involved in the downregulation of MMP14 expression, a target gene of miR-485-5p. UCA1 can be utilized as a prognostic biomarker and is connected to miR-485-5p and MMP14 in EOC metastasis [52].

Other lncRNAs related to ovarian cancer etiology

ZNF-300P1 could play a part in promoting metastasis in OvCa cells [30]. TC0100223, TC0101686 and TC0101441 are aberrantly expressed in estrogen receptor (ER) α -positive EOC tissues, showing correlations with advanced FIGO stage and/or high histological grade and lymph node metastasis. Multivariate analysis indicated that TC0101441 was also an independent prognostic factor for overall survival. Knowledge of these E2-regulated lncRNAs could aid in the future understanding of the estrogenic effect on EOC progression and could assist in the clinical design of new target therapies based on a perspective of lncRNA [45]. CCAT1, LOC645249, LOC100128881 and LOC100292680 were identified as metastasis-specific lncRNAs, in a cluster of seven lncRNAs, in a model comparing the parental SKOV3 and metastatic variant SKOV3.ip1 cells by microarray [20].

AB073614 promotes development of OvCa via targeting ERK1/2 and the AKT-mediated signaling pathway and is a poor prognostic marker for overall survival [13]. Major transcription factors binding on the AB073614 promoter are RUNX3, MTA3, EBF1, NF1C and ATF2 (as per ENCODE). MR22HG is a potential prognostic marker and functions as an oncogene in OvCa, its repression impaired migration, invasion, viability and downregulated the pro-metastatic gene, MYC, at the mRNA and protein level [36]. NEAT1 is upregulated in OvCa patients and cell lines, and its expression is associated with the FIGO stage and lymph node metastasis. NEAT1 is suppressed by miR-124-3p and could serve as a potential target for antineoplastic therapies [26]. NEAT1 expression level was an independent factor in predicting the overall survival of OvCa patients. NEAT1 also contributes to paclitaxel resistance of OvCa cells by regulating ZEB1 expression via miR-194 [8]. lncRNA AC104699.1.1 and RP11-284N8.3.1 are immune-associated, and were identified after co-expression network construction using molecular profiles of 399 OvCa patients. These were differentially expressed, were independently predictive of the survival of patients with different stages and have their predictive roles in immune system activation and other antitumor processes in the OvCa microenvironment [14]. Antisense noncoding RNA in the INK4 locus (ANRIL) overexpression correlated with advanced FIGO stage and high histological grade, indicative of independent prognostic factor for overall survival, lymph node metastasis and poor prognosis. The proliferative effect (cell cycle progression, inhibition of apoptosis and senescence) of ANRIL was linked to downregulation of P15INK4B and upregulation of Bcl-2 [15]. Colon-cancer-associated transcript (CCAT)2 is involved in several cancers, including OvCa. High CCAT2 expression levels are associated with a shorter overall survival and disease-free survival

in OvCa patients. CCAT2 expression positively correlates with the FIGO stage, tumor grade and distant metastasis [19]. Elevated levels of two different CRNDE transcripts were a negative prognostic factor; they increased the risk of death and recurrence in the group of patients treated with taxanes but not cyclophosphamide (DNA-damaging agents only) [21].

AL132709.8, HOTAIRM1, LOC100190986 and RUNX1-IT1 were identified as the OvCa recurrence signature to improve patient quality with personalized OvCa therapy [4]. Major transcription factors binding on the LOC100190986 promoter are Nkx3-1, HNF-1A, HNF-1, aMEF-2 and MEF-2A; and major transcription factors binding on the HOTAIRM1 promoter are PKNX1, ARNT, SIN3A, FEZF1 and ZNF2 (as per ENCODE). Analyzed in 266 fresh frozen tumor samples of EOC, ASAP1-IT1, FAM215A and LINC00472 are associated with patient survival. They were more frequently highly expressed in low-grade tumors and early-stage disease compared with high-grade tumors and late-stage disease [16].

XIST lncRNA correlates with levels and disease-free periods of OvCa patients with Taxol in the therapeutic regimens. XIST expression could be a potential marker for chemotherapeutic responses in OvCa [32]. Taurine upregulated gene (TUG)1 is upregulated in OvCa and positively correlates with tumor grade and the FIGO stage [34]. OVAAL lncRNA was identified by genomic analysis of GENCODE lncRNAs in high-grade serous ovarian adenocarcinoma. It is a potent regulator of cell physiology and tumor development [24]. NRCP is highly upregulated in ovarian tumors, and has a role in cancer metabolism, promotes cancer growth by altering glycolysis and elucidates functional effects leading to increased tumor progression as a binding partner of signal transducer and activator of transcription (STAT)1 [47]. Maternally expressed gene (MEG)3 lncRNA is epigenetically silenced in EOC owing to promoter hypermethylation, which could contribute to the development of EOC, it also activates p53 [48]. MEG3 regulates ATG3 and induces autophagy [51]. As a therapeutic approach, curcumin suppresses cisplatin resistance development partly via modulating extracellular vesicle-mediated transfer of MEG3 and miR-214 in OvCa [53]. lncRNA-HOST2 (human ovarian cancer-specific transcript 2) promotes tumor cell migration, invasion and proliferation in EOC through a mechanism involving miR-let-7b. HOST2 harbors a let-7b binding site and modulates let-7b availability by acting as a molecular sponge [29]. HGSC shows primary and acquired drug resistance toward cisplatin chemotherapy. Lin-RECK-3, H19, LUCAT1, LINC00961 and linc-CARS2-2 showed significantly increased expression levels in cisplatin-resistant A2780-DR cells, whereas Linc-TNFRSF19-1 and LINC00515 showed significantly decreased expression levels [54]. LSINCT5 is a 2.6 Kb polyadenylated, long stress-induced noncoding transcript, localized in the nucleus and potentially transcribed by RNA polymerase III. Overexpressed in OvCa, it regulates cellular proliferation. lncRNA NEAT-1 and a protein-coding gene PSCP1 were significantly affected by knockdown of LSINCT5 [49]. β -site APP-cleaving enzyme 1 antisense strand (BACE1-AS) identified as a novel target for anisomycin is responsible for OvCa stem cell (OCSC) proliferation and invasion [17]. Focally amplified lncRNA on chromosome 1 (FAL1) promotes its oncogenic activity (cancer cell growth) via repression of p21, and regulates the transcription of CDKN1A via stabilization of epigenetic repressor BMI1. It was identified in a genome-wide survey on somatic copy-number

alterations (SCNAs) of lncRNA in 2394 tumor specimens from 12 cancer types. *FAL1* is an oncogene, whose copy number and expression are correlated with outcomes in OvCa [18].

Growth-arrest-specific (GAS)5 lncRNA acts as a tumor suppressor in OvCa. When *GAS5* has decreased expression, this indicates poor prognosis in OvCa [62]. Lower *GAS5* expression in 60 EOC patients was closely related to lymph node metastasis and tumor node metastasis. *GAS5* also disrupts mitochondrial membrane potential, signifying its role in cell apoptosis through the mitochondria-mediated apoptosis pathway. It also promotes *BAX*, *BAK*, cleaved-caspase 3 and cleaved-caspase 9 expression. Overall, *GAS5* can serve as a novel therapeutic target in patients with EOC [63]. The homeobox A (*HOXA*) region of protein-coding genes impacts the female reproductive system embryogenesis and ovarian carcinogenesis. The 5' end of *HOXA* includes three lncRNAs: *HOXA10-AS*, *HOXA11-AS* and *HOTTIP*. GWAS data from 1201 serous EOC cases and 2009 controls identified *HOXA11-AS*, rs17427875 (*A* > *T*), which was marginally associated with reduced serous EOC risk. A functional variant of *HOXA11-AS*: *HOXA11-AS* minor allele-*T*, inhibits the oncogenic phenotype of EOC, compared with common allele-*A* expression in EOC. *HOXA11-AS* expression levels were significantly lower in human EOC tumors than normal ovarian tissues, suggesting a tumor suppressor function enhanced by the *T* allele [46]. *BCYRNA* or *BC200* RNA downregulation leads to cancer cell proliferation and chemoresistance to carboplatin in *SKOV3* and *A2780* cells, it appears to have a role in the mediation of carboplatin-induced OvCa cell death [22].

Concluding remarks and future directions

OvCa is the leading cause of death among all gynecological malignancies. lncRNA has given a new facet to OvCa, to date not many lncRNAs reported have been functionally characterized in the OvCa disease process. Many of the lncRNAs have been identified by revisiting the array datasets on the publicly available resources, making it necessary to understand the mechanisms and biology of this new class of regulators, which is now at the center of various physiological and

pathological processes. The information regarding OvCa-specific lncRNAs either alone or in combination with other types of markers (miRNAs, mRNAs, proteins) could prove useful to predict outcome or treatment follow-up to improve the therapeutic care of ovarian carcinoma patients. At present, more research is needed to elucidate the biological mechanisms [64] and clinical implications in tumor characterization as well as disease prognosis and treatment at the molecular level, putting them into clinical practice.

The field of precision medicine is getting traction and it would need analysis and identification of novel factors responsible for complex diseases like OvCa. The information about the current state of new lncRNAs reported in OvCa will help to identify new approaches to develop new therapies based on lncRNA status. lncRNAs are an attractive biomarker considering their expression pattern, tissue specificity and detectability using a small amount of sample. This comprehensive review summarizes the current state of new lncRNAs reported in OvCa along with the accessible information on their genomic locus and regulating transcription factors. This crucial information can be used to design therapeutics based on 'silencing' using siRNAs, antisense oligos, ribozymes, CRISPR, ZNFs and TALENs or 'functional inhibition' using small molecules, nanobodies, aptamers and RNA decoys for lncRNA therapeutics.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgments

This work was supported by the National Institutes of Health Grant (R01CA204552, R01CA210192 and R01CA206069) R01CA204552, R01CA210192 and R01CA206069 to S.C.C. UTHSC-CORNET and UTHSC-Clinical CORNET award to M.K.T. This work was also partially supported by UTHSC-College of Pharmacy-Dean's Seed Grant and UTHSC New Grant Mechanism Award to S.C.C., M.M.Y. and M.J. Also partially supported by K22CA174841 and CA213232 grants to MMY.

References

- 1 ENCODE Project Consortium (2012) An integrated encyclopedia of DNA elements in the human genome. *Nature* 489, 57–74
- 2 Zhan, X. *et al.* (2017) Panel of seven long noncoding RNA as a candidate prognostic biomarker for ovarian cancer. *Oncotargets Ther.* 10, 2805–2813
- 3 Liu, R. *et al.* (2017) Long noncoding RNA expression signature to predict platinum-based chemotherapeutic sensitivity of ovarian cancer patients. *Sci. Rep.* 7, 18
- 4 Yang, K. *et al.* (2017) Identification of a six-lncRNA signature associated with recurrence of ovarian cancer. *Sci. Rep.* 7, 752
- 5 Prat, J. (2015) FIGO's staging classification for cancer of the ovary, fallopian tube, and peritoneum: abridged republication. *J. Gynecol. Oncol.* 26, 87–89
- 6 Coleman, R.L. *et al.* (2013) Latest research and treatment of advanced-stage epithelial ovarian cancer. *Nat. Rev. Clin. Oncol.* 10, 211–224
- 7 Fu, L.L. *et al.* (2017) Role of lncRNAs as novel biomarkers and therapeutic targets in ovarian cancer. *Crit. Rev. Eukaryot. Gene Expr.* 27, 183–195
- 8 Wang, Q. *et al.* (2017) Long noncoding RNAs (lncRNAs) in triple negative breast cancer. *J. Cell. Physiol.* 232, 3226–3233
- 9 Quan, M. *et al.* (2015) Exploring the secrets of long noncoding RNAs. *Int. J. Mol. Sci.* 16, 5467–5496
- 10 Ma, L. *et al.* (2013) On the classification of long non-coding RNAs. *RNA Biol.* 10, 925–933
- 11 Rinn, J.L. and Chang, H.Y. (2012) Genome regulation by long noncoding RNAs. *Annu. Rev. Biochem.* 81, 145–166
- 12 Gutschner, T. *et al.* (2018) From biomarkers to therapeutic targets—the promises and perils of long non-coding RNAs in cancer. *Cancer Metastasis Rev.* 37, 83–105
- 13 Cheng, Z. *et al.* (2015) A long noncoding RNA AB073614 promotes tumorigenesis and predicts poor prognosis in ovarian cancer. *Oncotarget* 6, 25381–25389
- 14 Guo, Q. *et al.* (2015) Comprehensive analysis of lncRNA-mRNA co-expression patterns identifies immune-associated lncRNA biomarkers in ovarian cancer malignant progression. *Sci. Rep.* 5, 17683
- 15 Qiu, J.J. *et al.* (2016) The long non-coding RNA ANRIL promotes proliferation and cell cycle progression and inhibits apoptosis and senescence in epithelial ovarian cancer. *Oncotarget* 7, 32478–32492
- 16 Fu, Y. *et al.* (2016) Long non-coding RNAs, *ASAP1-IT1*, *FAM215A*, and *LINC00472*, in epithelial ovarian cancer. *Gynecol. Oncol.* 143, 642–649
- 17 Chen, Q. *et al.* (2016) Long non-coding RNA *BACE1-AS* is a novel target for anisomycin-mediated suppression of ovarian cancer stem cell proliferation and invasion. *Oncol. Rep.* 35, 1916–1924
- 18 Hu, X. *et al.* (2014) A functional genomic approach identifies *FAL1* as an oncogenic long noncoding RNA that associates with *BMI1* and represses *p21* expression in cancer. *Cancer Cell* 26, 344–357
- 19 Huang, S. *et al.* (2016) The long non-coding RNA *CCAT2* is up-regulated in ovarian cancer and associated with poor prognosis. *Diagn. Pathol.* 11, 49
- 20 Liu, S.P. *et al.* (2013) Identification of differentially expressed long non-coding RNAs in human ovarian cancer cells with different metastatic potentials. *Cancer Biol. Med.* 10, 138–141
- 21 Szafron, L.M. *et al.* (2015) The putative oncogene, *CRNDE*, is a negative prognostic factor in ovarian cancer patients. *Oncotarget* 6, 43897–43910

- 22 Wu, D.I. *et al.* (2016) Downregulation of BC200 in ovarian cancer contributes to cancer cell proliferation and chemoresistance to carboplatin. *Oncol. Lett.* 11, 1189–1194
- 23 Zhou, M. *et al.* (2016) Comprehensive analysis of lncRNA expression profiles reveals a novel lncRNA signature to discriminate nonequivalent outcomes in patients with ovarian cancer. *Oncotarget* 7, 32433–32448
- 24 Akrami, R. *et al.* (2013) Comprehensive analysis of long non-coding RNAs in ovarian cancer reveals global patterns and targeted DNA amplification. *PLoS One* 8, e80306
- 25 An, J. *et al.* (2017) LncRNA NEAT1 contributes to paclitaxel resistance of ovarian cancer cells by regulating ZEB1 expression via miR-194. *Onco Targets Ther.* 10, 5377–5390
- 26 Chai, Y. *et al.* (2016) HuR-regulated lncRNA NEAT1 stability in tumorigenesis and progression of ovarian cancer. *Cancer Med.* 5, 1588–1598
- 27 Chen, Q. *et al.* (2016) Plasma long non-coding RNA MALAT1 is associated with distant metastasis in patients with epithelial ovarian cancer. *Oncol. Lett.* 12, 1361–1366
- 28 Cui, L. *et al.* (2013) Expression of long non-coding RNA HOTAIR mRNA in ovarian cancer. *Sichuan Da Xue Xue Bao Yi Xue Ban* 44, 57–59
- 29 Gao, Y. *et al.* (2015) LncRNA-HOST2 regulates cell biological behaviors in epithelial ovarian cancer through a mechanism involving microRNA let-7b. *Hum. Mol. Genet.* 24, 841–852
- 30 Gloss, B. *et al.* (2014) ZNF300P1 encodes a lincRNA that regulates cell polarity and is epigenetically silenced in type II epithelial ovarian cancer. *Mol. Cancer* 13, 3
- 31 Guan, Y. *et al.* (2007) Amplification of PVT1 contributes to the pathophysiology of ovarian and breast cancer. *Clin. Cancer Res.* 13, 5745–5755
- 32 Huang, K.C. *et al.* (2002) Relationship of XIST expression and responses of ovarian cancer to chemotherapy. *Mol. Cancer Ther.* 1, 769–776
- 33 Jin, Y. *et al.* (2017) LncRNA MALAT1 promotes proliferation and metastasis in epithelial ovarian cancer via the PI3K-AKT pathway. *Eur. Rev. Med. Pharmacol. Sci.* 21, 3176–3184
- 34 Kuang, D. *et al.* (2016) Long non-coding RNA TUG1 regulates ovarian cancer proliferation and metastasis via affecting epithelial-mesenchymal transition. *Exp. Mol. Pathol.* 101, 267–273
- 35 Li, J. *et al.* (2016) Overexpression of long non-coding RNA HOTAIR leads to chemoresistance by activating the Wnt/beta-catenin pathway in human ovarian cancer. *Tumour Biol.* 37, 2057–2065
- 36 Li, J. *et al.* (2016) Long noncoding RNA C17orf91 is a potential prognostic marker and functions as an oncogene in ovarian cancer. *J. Ovarian Res.* 9, 49
- 37 Liu, E. *et al.* (2015) Carboplatin-docetaxel-induced activity against ovarian cancer is dependent on up-regulated lncRNA PVT1. *Int. J. Clin. Exp. Pathol.* 8, 3803–3810
- 38 Liu, E. *et al.* (2015) Overexpression of long non-coding RNA PVT1 in ovarian cancer cells promotes cisplatin resistance by regulating apoptotic pathways. *Int. J. Clin. Exp. Med.* 8, 20565–20572
- 39 Mizrahi, A. *et al.* (2009) Development of targeted therapy for ovarian cancer mediated by a plasmid expressing diphtheria toxin under the control of H19 regulatory sequences. *J. Transl. Med.* 7, 69
- 40 Ozes, A.R. *et al.* (2016) NF-kappaB-HOTAIR axis links DNA damage response, chemoresistance and cellular senescence in ovarian cancer. *Oncogene* 35, 5350–5361
- 41 Pa, M. *et al.* (2018) Long noncoding RNA MALAT1 functions as a sponge of MiR-200c in ovarian cancer. *Oncol. Res.* <http://dx.doi.org/10.3727/096504017X15049198963076> in press
- 42 Qiu, J. *et al.* (2014) Effects of oestrogen on long noncoding RNA expression in oestrogen receptor alpha-positive ovarian cancer cells. *J. Steroid Biochem. Mol. Biol.* 141, 60–70
- 43 Qiu, J.J. *et al.* (2014) Overexpression of long non-coding RNA HOTAIR predicts poor patient prognosis and promotes tumor metastasis in epithelial ovarian cancer. *Gynecol. Oncol.* 134, 121–128
- 44 Qiu, J.J. *et al.* (2015) The long non-coding RNA HOTAIR promotes the proliferation of serous ovarian cancer cells through the regulation of cell cycle arrest and apoptosis. *Exp. Cell Res.* 333, 238–248
- 45 Qiu, J.J. *et al.* (2014) Expression and clinical significance of estrogen-regulated long non-coding RNAs in estrogen receptor alpha-positive ovarian cancer progression. *Oncol. Rep.* 31, 1613–1622
- 46 Richards, E.J. *et al.* (2015) A functional variant in HOXA11-AS, a novel long non-coding RNA, inhibits the oncogenic phenotype of epithelial ovarian cancer. *Oncotarget* 6, 34745–34757
- 47 Rupaimoole, R. *et al.* (2015) Long noncoding RNA ceruloplasmin promotes cancer growth by altering glycolysis. *Cell Rep.* 13, 2395–2402
- 48 Sheng, X. *et al.* (2014) Promoter hypermethylation influences the suppressive role of maternally expressed 3, a long non-coding RNA, in the development of epithelial ovarian cancer. *Oncol. Rep.* 32, 277–285
- 49 Silva, J.M. *et al.* (2011) LSINCT5 is over expressed in breast and ovarian cancer and affects cellular proliferation. *RNA Biol.* 8, 496–505
- 50 Wang, F. *et al.* (2015) Involvement of SRPK1 in cisplatin resistance related to long non-coding RNA UCA1 in human ovarian cancer cells. *Neoplasma* 62, 432–438
- 51 Xiu, Y.L. *et al.* (2017) Upregulation of the lncRNA Meg3 induces autophagy to inhibit tumorigenesis and progression of epithelial ovarian carcinoma by regulating activity of ATG3. *Oncotarget* 8, 31714–31725
- 52 Yang, Y. *et al.* (2016) UCA1 functions as a competing endogenous RNA to suppress epithelial ovarian cancer metastasis. *Tumour Biol.* 37, 10633–10641
- 53 Zhang, J. *et al.* (2017) Curcumin suppresses cisplatin resistance development partly via modulating extracellular vesicle-mediated transfer of MEG3 and miR-214 in ovarian cancer. *Cancer Chemother. Pharmacol.* 79, 479–487
- 54 Zheng, Z.G. *et al.* (2016) The essential role of H19 contributing to cisplatin resistance by regulating glutathione metabolism in high-grade serous ovarian cancer. *Sci. Rep.* 6, 26093
- 55 Zhou, Y. *et al.* (2016) The long noncoding RNA MALAT-1 is highly expressed in ovarian cancer and induces cell growth and migration. *PLoS One* 11, e0155250
- 56 Zhu, Z. *et al.* (2015) Ectopic expressed long non-coding RNA H19 contributes to malignant cell behavior of ovarian cancer. *Int. J. Clin. Exp. Pathol.* 8, 10082–10091
- 57 Zou, A. *et al.* (2016) Long non-coding RNA MALAT1 is up-regulated in ovarian cancer tissue and promotes SK-OV-3 cell proliferation and invasion. *Neoplasma* 63, 865–872
- 58 Meryet-Figuere, M. *et al.* (2016) An overview of long non-coding RNAs in ovarian cancers. *Oncotarget* 7, 44719–44734
- 59 Nikpayam, E. *et al.* (2017) The role of long non-coding RNAs in ovarian cancer. *Iran Biomed. J.* 21, 3–15
- 60 Lei, R. *et al.* (2017) Long noncoding RNA MALAT1-regulated microRNA 506 modulates ovarian cancer growth by targeting iASPP. *Onco Targets Ther.* 10, 35–46
- 61 Wilusz, J.E. (2016) Long noncoding RNAs: re-writting dogmas of RNA processing and stability. *Biochim. Biophys. Acta* 1859, 128–138
- 62 Li, J. *et al.* (2016) Decreased expression of long non-coding RNA GASS promotes cell proliferation, migration and invasion, and indicates a poor prognosis in ovarian cancer. *Oncol. Rep.* 36, 3241–3250
- 63 Gao, J. *et al.* (2015) Long non-coding RNA growth arrest-specific transcript 5 is involved in ovarian cancer cell apoptosis through the mitochondria-mediated apoptosis pathway. *Oncol. Rep.* 34, 3212–3221
- 64 Kung, J.T. *et al.* (2013) Long noncoding RNAs: past, present, and future. *Genetics* 193, 651–669