Microarray analysis reveals differentially expressed lncRNAs in benign epithelial ovarian cysts and normal ovaries

XIAOGUANG LIU1,2*, CHENCHENG DAI1,3*, GENMEI JIA1,3, SUJUAN XU4, ZIYI FU1, JUAN XU1,2, QING LI5, HONGJIE RUAN2 and PENGFEI XU1

1Nanjing Maternal and Child Health Institute, and 2Department of Gynecology, Nanjing Maternal and Child Health Care Hospital, Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University, Nanjing, Jiangsu 210004; 3The First Clinical Medical College of Nanjing Medical University, Nanjing, Jiangsu 210029; 4Department of Clinical Laboratory, Nanjing Maternal and Child Health Care Hospital, Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University, Nanjing, Jiangsu 210004; 5Department of Pathology, Shanghai Pudong New Area People's Hospital, Shanghai 201040, P.R. China

Received December 28, 2016; Accepted June 9, 2017

DOI: 10.3892/or.2017.5741

Abstract. Recent studies suggest that long non-coding RNAs (lncRNAs) play crucial roles in many types of human malignant cancers. However, the function of lncRNAs in benign tumors remains poorly understood. In the present study, to explored the potential roles of lncRNAs in benign epithelial ovarian cysts (BEOCs) which commonly occur in young women and possess malignant potential, we described the expression profile of the lncRNAs between BEOC and normal ovarian tissues using lncRNA microarray techniques. The results showed that 1,325 transcripts of lncRNAs (1,014 upregulated and 311 downregulated) were differentially expressed in the BEOCs compared with the normal controls [absolute fold-change ≥2, false discovery rate (FDR) <0.05]. We also conducted quantitative real-time PCR (qPCR) to confirm the microarray data. The results of qPCR revealed that the expression trend of 6 randomly selected lncRNAs was consistent with the microarray data. Furthermore, candidate lncRNAs were characterized by pathway analysis and Gene Ontology (GO). The present study is the first to demonstrate different expression profiles of lncRNAs between BEOCs and normal ovarian tissues. These lncRNAs may play a crucial role in the pathological process of BEOCs.

Introduction

Ovarian tumors are the leading cause of death among all gynecologic tumors (1). In the US, ~22,280 cases of ovarian cancer were estimated to be diagnosed and it was estimated that 14,240 women died from ovarian cancer in 2016 (2). Despite modern surgical techniques and chemotherapy, the prognosis of ovarian cancer remains poor (3). Ovarian tumors are a common type of neoplasm in women and the histological type is various and complex. Epithelial ovarian tumors are the most common type of ovarian tumor and represents 50-70% of all primary ovarian tumors. According to the characteristics of the tumor cells and the severity to health, epithelial ovarian tumors can be divided into three types: benign, borderline and malignant ovarian tumors. In various cases, benign tumors can develop into a malignant tumor (4-7), which suggests that benign ovarian tumors have an increased risk to transform into malignant tumors due to the changes of various genes (8) or proteins (9). However, extensive research has mainly focused on malignant epithelial ovarian cancer, and benign ovarian tumors have not been a principal focus of research. In fact, there are far more benign lesions occurring in the epithelial ovary, and these are commonly diagnosed during pregnancy (10). In addition, these are also associated with an increased risk of malignant epithelial ovarian cancer. Therefore, a deeper understanding of benign epithelial ovarian cysts (BEOCs) may not only provide more effective treatments for BEOCs, but may also reduce the incidence of malignant epithelial ovarian cancer.

Long non-coding RNAs (lncRNAs) are a type of RNAs that are longer than 200 nucleotides in length and without coding protein capacity (11). lncRNAs have been previously considered as ‘transcriptional noise’ for a long time (12). Recently, studies have confirmed that lncRNAs play a critical role in the development of cancer, including ovarian cancer. Emerging
evidence suggests that lncRNAs are associated with ovarian cancer biological behaviors such as cell proliferation (13,14), apoptosis (15,16), and invasion and metastasis (17,18). Moreover, due to tissue-specificity, some lncRNAs may served as potential biomarkers for cancer prognosis, including ovarian cancer (19). However, these studies have only focused on the regulation of lncRNAs in malignant ovarian cancer, and little research has been carried out on the relationship between lncRNAs and benign ovarian tumors. Therefore, lncRNA expression profiles in BEOCs may help us to better understand BEOC pathogenesis.

In the present study, we described the distinct expression profiles of lncRNAs in BEOC and normal ovarian epithelial tissues. In total, 1,014 transcripts of lncRNAs were upregulated and 311 transcripts of lncRNAs were downregulated in BEOCs compared with normal controls [absolute fold-change ≥2, false discovery rate (FDR) <0.05]. Moreover, we also examined the Gene Ontology (GO) enrichment of their associated protein-coding genes and performed pathway analyses to analyze the potential function of these differentially expressed lncRNAs. The present study may aid in elucidating the tumorigenesis of ovarian epithelial tissue and decrease the incidence of malignant transformation in regards to BEOCs.

Materials and methods

Tissue collection. Samples of BEOCs and normal ovarian tissues were collected from the Gynecologic Oncology Department of Nanjing Maternal and Child Health Hospital (Nanjing, China) from 2014 to 2016. No patient received chemotherapy or radiotherapy before surgery. Informed consent for the use of the tissues was obtained from all patients. Finally, 10 cases of normal ovarian tissues and 17 cases of BEOCs were collected and immediately stored in RNAsafety™ and frozen at -80°C before the experiments. All the tissues were confirmed through histopathological diagnoses. The present study was approved by the Ethics Review Committee of Nanjing Maternity and Child Health Care Hospital.

Total RNA extraction. Frozen tissues were dissolved in TRIZol reagent (Life Technologies, Grand Island, NY, USA). Total RNA was extracted according to the manufacturer’s protocol (Invitrogen, Carlsbad, CA, USA). NanoDrop and the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) were used to check the quantification and quality of the extracted RNA, respectively. The extracted RNA samples were stored at -80°C for further experiments. Complementary DNA (cDNA) was synthesized from 1 μg of total-RNA using a Primescript™ RT Master Mix kit (Applied Takara, Dalian, China) with random hexamer primers in a final volume of 20 ml. The condition of reverse-transcription reaction was as follows: 37°C for 15 min, 85°C for 5 sec and 4°C for 10 min.

Microarray analysis. To screen the global profiling of human lncRNAs and protein-coding transcripts, we profiled three BEOC tissues and three normal ovarian epithelial tissues with ArayStar Human Microarray V3.0. The lncRNAs were searched for using authoritative databases such as RefSeq, Ensembl and UCSC Known Genes and related studies while the mRNAs were collected from RefSeq and GENCODE. To recognize every individual transcript exactly, each transcript was described with a specific exon or splice junction probe. Both positive probes (the housekeeping genes) and negative probes were printed onto the array for hybridization quality control. The sample processing and microarray hybridization were performed in terms of the Agilent One-Color Microarray-Based Gene Expression Analysis Protocol (Agilent Technology). Briefly, 1 mg of total RNA was obtained for purification by removing the rRNA (mRNA-ONLY Eukaryotic mRNA Isolation kit; Epicentre, Madison, WI, USA). Then, each sample was amplified and transcribed into fluorescent cRNA along the entire length of the transcripts without 3’ bias utilizing a random priming method. Agilent Quick Amp Labeling kit was employed to normalize the values, and then, lncRNAs and mRNAs, for which at least one out of two groups had flags in present or marginal, were chosen for further data analysis. Additionally, hierarchical clustering and combined analyses were performed using homemade scripts.

Quantitative real-time PCR (qPCR). qPCR was performed to detect the relative gene expression using Power SYBR-Green PCR Master Mix (2X Applied Biosystems) according to the standard protocol. GAPDH was taken as an internal reference. The qPCR reaction conditions were set as follows: an initial denaturation at 95°C for 30 sec, followed by 40 PCR cycles at 95°C for 5 sec and 60°C for 34 sec. Finally annealing and extension at 95°C for 15 sec, 60°C for 60 sec and 95°C for 15 sec. Each sample was detected in triplicates. The fold-change was calculated with the ΔCT method to describe the relative gene expression in BEOC samples relative to the normal ovarian tissue samples. All of the primers are presented in Table I.

GO and pathway analyses. Pathway and GO analyses were applied to determine the potential roles of differentially expressed lncRNAs in biological pathways or GO terms. The predicted target genes of the differentially expressed lncRNAs were mapped to GO terms in the Database for Annotation Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov/). Fisher’s exact test was used to ascertain whether true differences existed between the groups. In addition, we used the Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.kegg.jp/) to confirm the pathway enrichment analysis. The ontology covers three domains: biological process (BP), cellular component (CC) and molecular function (MF). The threshold of significance was defined by FDR.

Statistical analysis. Differential expression levels of lncRNAs were selected by fold-change filtering (absolute fold-change ≥2.0). Independent samples t-test between two groups was used, and Fisher’s exact test was used in GO and pathway analyses. A value of FDR <0.05 was considered statistically significant. Computer-based calculations were conducted using SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA).

Results

Differentially expressed lncRNAs and mRNAs in BEOCs compared with normal ovarian tissues. Firstly, to explore the
altered lncRNAs in the BEOCs, we determined the lncRNA and mRNA expression profiles using microarray analyses of normal ovarian and BEOC tissues. Heatmaps and scatter-plots were used to describe the variation in lncRNA expression among normal ovarian, benign ovarian cysts and malignant epithelial ovarian cancer tissues (Fig. 1). All lncRNAs and mRNAs with a signal altered by 2-fold and with a false discovery rate (FDR) <0.05 were identified as statistically altered. Finally, 1,325 transcripts of lncRNAs (1,014 upregulated and 311 downregulated) and 1,563 mRNAs (613 upregulated and 950 downregulated) were found to be differentially expressed in BEOC tissues compared with normal controls. Lists of the top 20 upregulated and downregulated lncRNAs identified in the microarray analyses are presented in Tables II and III.
| Seqname               | GeneSymbol       | FDR   | Fold-change | Source       | Associated-gene | Mean expression |
|----------------------|------------------|-------|-------------|--------------|-----------------|-----------------|
| ENST00000502882      | RP11-158J3.2     | 0.017 | 44.223      | GENCODE      |                 | 38.428          |
| ENST00000534866      | TAS2R64P         | 0.022 | 42.733      | GENCODE      |                 | 5.029           |
| uc010ciy.1           | BC160930         | 0.038 | 41.621      | UCSC_knowngene | RP11-566K11.2  | 7.028           |
| AK021689             |                  | 0.017 | 40.678      | NRED         |                 | 19.848          |
| ENST00000504047      | RP11-32B5.7      | 0.031 | 40.156      | GENCODE      |                 | 9.524           |
| ENST00000419463      | ACO19117.1       | 0.018 | 33.387      | GENCODE      |                 | 5.110           |
| ENST00000526388      | CTC-497E21.4     | 0.010 | 28.260      | GENCODE      |                 | 8.881           |
| ENST00000563752      | SLC25A3P1        | 0.019 | 26.511      | GENCODE      |                 | 5.029           |
| ENST00000566892      | RP11-1081M5.2    | 0.027 | 21.685      | GENCODE      |                 | 26.929          |
| NR_040017            | RNF157-AS1       | 0.020 | 21.544      | RefSeq       | FOXJ1           | 6.342           |
| uc001gzi.3           | BC034684         | 0.005 | 17.922      | UCSC_knowngene | CHI3L1         | 5.029           |
| ENST00000450480      | RP4-797C5.2      | 0.018 | 17.793      | GENCODE      | KCND2           | 8.340           |
| TCONS_00026830       | XLOC_013047      | 0.034 | 16.688      | LincRNAs identified by Cabili et al (12) | | 7.306 |
| NR_040033            | LOC729950        | 0.023 | 16.490      | RefSeq       |                 | 37.444          |
| NR_027309            | LOC148824        | 0.016 | 16.354      | RefSeq       | OR2C3           | 68.207          |
| ENST00000381181      | AP000569.2       | 0.034 | 16.383      | GENCODE      |                 | 12.908          |
| NR_0283883           | LOC000649        | 0.017 | 16.086      | RefSeq       |                 | 28.062          |
| TCONS_00018520       | XLOC_008826      | 0.021 | 15.539      | LincRNAs identified by Cabili et al (12) | | 5.811 |
| chr14:84031800-84050525+ | chr14:84031800-84050525 | 0.024 | 15.300      | LincRNAs identified by Khalil et al (26) | | 5.029 |

IncRNAs, long non-coding RNAs; FDR, false discovery rate.
Table III. The top 20 downregulated lncRNAs in the BEOCs compared with the normal ovarian tissues.

| Seqname       | Genesymbol   | FDR   | Fold-change | Source               | Associated-gene | Mean expression |
|---------------|--------------|-------|-------------|----------------------|-----------------|-----------------|
| uc002ejp.1    | mT1JP        | 0.016 | 58.627      | UCSC_knowngene       | 1999.563        | 34.107          |
| uc003xxw.1    | AX747593     | 0.020 | 24.817      | UCSC_knowngene       | 215.542         | 8.685           |
| ENST00000584923 | SNORD3 A  | 0.014 | 20.002      | GENCODE              | 5117.821        | 255.872         |
| ENST00000437593 | RP11-500G22.2 | 0.012 | 18.427      | GENCODE              | 215.542         | 8.685           |
| TCONS_00023858 | XLOC_011173 | 0.014 | 16.438      | LincRNAs identified by Cabili et al (12) | 432.820         | 26.330          |
| ENST00000542078 | RP11-392P7.8 | 0.014 | 15.472      | GENCODE              | 1999.563        | 34.107          |
| ENST00000417522 | RP11-382J22.6 | 0.017 | 13.893      | GENCODE              | 70.787          | 5.095           |
| ENST00000580684 | RP11-835E18.2 | 0.048 | 13.632      | GENCODE              | 154.759         | 11.353          |
| ENST00000536029 | RP11-392P7.8  | 0.020 | 11.776      | GENCODE              | 113.762         | 9.660           |
| TCONS_00017618 | XLOC_008306  | 0.011 | 11.503      | LincRNAs identified by Cabili et al (12) | 635.853         | 55.276          |
| uc002kch.1    | AK095045     | 0.018 | 10.279      | UCSC_knowngene       | 335.169         | 32.609          |
| TCONS_00014161 | XLOC_006144  | 0.023 | 10.191      | LincRNAs identified by Cabili et al (12) | 178.103         | 17.477          |
| ENST00000499314 | RP11-1277A3.2 | 0.021 | 10.179      | GENCODE              | 522.572         | 51.336          |
| TCONS_00029245 | XLOC_013980  | 0.031 | 10.076      | LincRNAs identified by Cabili et al (12) | 294.817         | 29.260          |
| ENST00000417089 | H19         | 0.017 | 9.329       | GENCODE              | 508.334         | 54.491          |
| ENST00000555882 | DIO3OS      | 0.005 | 9.060       | GENCODE              | 46.162          | 5.095           |
| NR_026860     | LINCO00473   | 0.020 | 8.979       | RefSeq               | 410.373         | 45.702          |
| uc010rog.2    | NEAT1        | 0.044 | 8.809       | UCSC_knowngene       | 261.236         | 29.656          |
| ENST00000520913 | PVT1        | 0.010 | 8.783       | GENCODE              | 1881.694        | 214.238         |
| ENST00000447298 | H19         | 0.017 | 8.718       | GENCODE              | 688.447         | 78.972          |

lncRNAs, long non-coding RNAs; FDR, false discovery rate.
Validation of candidate lncRNAs by qPCR. To confirm the validity of the microarray data, we next conducted qPCR to detect the expression of the lncRNAs. We randomly selected 6 differentially expressed lncRNAs. Among the 6 lncRNAs, lncRNAs LOC339166, LOC441204, LOC644656 and NENF were upregulated whereas lncRNAs RP11-471J12.1 and mEG3 were downregulated in the BEOCs compared with the normal controls (Fig. 2). The result of qPCR confirmed that the expression trend of the 6 selected lncRNAs was consistent with the microarray data.

GO and pathway analyses of the differentially expressed lncRNAs. To investigate the function of altered lncRNAs in the BEOCs, we performed GO analysis which covered the following three domains: biological processes (BP), cellular components (CC) and molecular functions (MF). We found that the highest GO classifications targeted by the upregulated lncRNAs were single-organism process (Fig. 3A), membrane (Fig. 3B) and signal transducer activity (Fig. 3C). However, the highest GO classifications targeted by downregulated lncRNAs were cellular process (Fig. 3D), cell part (Fig. 3E) and binding, particularly protein binding (Fig. 3F). To map these lncRNAs to pathways, we also performed pathway analysis. The result indicated that the 9 main pathways corresponding to the upregulated transcripts and the most enriched network was 'neuroactive ligand-receptor interaction' (Fig. 4A). The 9 main pathways are shown: i) neuroactive ligand-receptor interaction; ii) axon guidance; iii) mucin type O-glycan biosynthesis; iv) Staphylococcus aureus infection; v) transcriptional misregulation in cancer; vi) GABAergic synapse; vii) carbohydrate digestion and absorption; viii) serotonergic synapse; ix) glutamatergic synapse. We also observed 10 main pathways corresponding to the downregulated transcripts (Fig. 4B): i) focal adhesion; ii) bacterial invasion of epithelial cells; iii) phagosome; iv) leukocyte transendothelial migration;
Figure 3. GO analysis of the differentially expressed lncRNAs. GO analysis results show the differentially expressed lncRNAs associated with biological processes (BP), cellular components (CC) and molecular functions (MF). The most frequent fold enrichment BP associated with (A) upregulated lncRNAs and (D) downregulated lncRNAs in BEOCs. The most frequent fold enrichment CC for (B) lncRNAs upregulated in BEOCs compared with normal control. The top 10 GO terms of MF associated with (C) upregulated lncRNAs.
v) viral myocarditis; vi) pathogenic *Escherichia coli* infection; vii) steroid biosynthesis; viii) malaria; ix) leishmaniasis; x) *Vibrio cholera* infection. The most enriched network was ‘focal adhesion’ with 37 transcripts annotated with this term (Fisher P-value=1.53367E-06).

**Discussion**

BEOCs are the most common form of ovarian tumors in women, accounting for ~80% of all ovarian masses (20). However, the molecular mechanisms related to the tumorigenesis of ovarian epithelial cells remains largely unknown. Increasing studies have claimed that lncRNAs are highly functional and have a crucial role in malignant ovarian cancer (13,17,18,21,22). In our previous study, we identified dysregulation of many lncRNAs in malignant ovarian cancer compared with benign ovarian cysts and normal ovary. However, we also found that several lncRNAs, such as LEMD1-AS1 and AK 125532 were differentially expressed in BEOCs compared with these in the normal control (23). Thus, we next identified differentially expressed lncRNAs in BEOCs compared with normal ovarian tissues. To the best of our knowledge, the present study is the...
first to investigate the lncRNA expression profiling in BEOCs compared with normal ovarian tissues. The present study provides a better understanding of the molecular mechanisms of BEOCs.

On the basis of the GO analysis, we found that the BP of upregulated and downregulated lncRNAs was both tightly associated with single-organism process and single-organism cellular process. These GO terms are also associated with death and cell proliferation (24). The destiny of BEOCs is critically regulated by the cell cycle and apoptosis process in which lncRNAs may play an important role. For CC, the top GO term of the upregulated lncRNAs was membrane part and in downregulated lncRNAs this was cell part. These results indicate that lncRNAs may primarily regulate various mRNAs which are located in the cell or on the membrane and exercise their function. It can also be observed that the highest frequency of the MF GO terms in the upregulated lncRNAs was molecular transducer activity. Many studies have reported that some lncRNAs act as a molecular transducer. For example, LncRNA-TuG1 is overexpressed in non-small cell lung cancer, and can be regulated by p53 and affect cell proliferation through HOXB7 expression (25). However, the top MF GO term in the downregulated lncRNAs was binding, particularly protein binding. Increasing studies suggest that the primary function of lncRNAs is the epigenetic regulation of coding gene through proteins or microRNAs. Many lncRNAs have been reported to recruit and bind to PRC2 (26) or other chromatin-associated proteins (27). In addition, accumulating evidence indicates that lncRNAs act as competing endogenous RNAs (ceRNAs) by ‘sponging’ microRNAs (22,28,29).

In addition, pathway analysis displayed that the upregulated lncRNAs were mainly correlated with focal adhesion. Focal neural signaling pathways. In contrast, the downregulated lncRNAs were mainly correlated with neuroactive RNAs (ceRNAs) by ‘sponging’ microRNAs (22,28,29).

Acknowledgements

The present study was supported by the National Natural Science Foundation of China (grant no. 81402139).

References

1. Holtschneider CH and Berek JS: Ovarian cancer: Epidemiology, biology, and prognostic factors. Semin Surg Oncol 19: 3-10, 2000.
2. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2016. CA Cancer J Clin 66: 7-30, 2016.
3. Cheng YC, Chen CA, Chiang CJ, Hsu TH, Lin MC, You SL, Cheng WF and Lai MS: Trends in incidence and survival outcome of epithelial ovarian cancer: 30-Year national population-based registry in Taiwan. J Gynecol Oncol 24: 342-351, 2013.
4. Austin RM: Benign to malignant transformation in epithelial ovarian tumors. Hum Pathol 24: 562-563, 1993.
5. Fitzgibbons PL, Henson DE and Hutter RV: Cancer Committee of the College of American Pathologists: Benign breast changes and the risk for subsequent breast cancer: An update of the 1985 consensus statement. Arch Pathol Lab Med 122: 1053-1055, 1998.
6. Dupont WD, Page DL, Parli FF, Vnencak-Jones CL, Plummer WD Jr, Rados MS and Schuyler PA: Long-term risk of breast cancer in women with fibroadenoma. N Engl J Med 331: 10-15, 1994.
7. McDivitt RW, Stevens JA, Lee NC, Wingo PA, Rubin GL and Gersell D; The Cancer and Steroid Hormone Study Group: Histologic types of benign breast disease and the risk for subsequent breast cancer: An update of the 1985 consensus statement. Arch Pathol Lab Med 122: 1053-1055, 1998.
8. Powell DE, Puls L and van Nagell J Jr: Current concepts in epithelial ovarian tumors: Does benign to malignant transformation occur? Hum Pathol 23: 846-847, 1992.
9. Waldemarson S, Krog M, Alaya A, Kirik U, Schedvins K, Auer G, Hansson KM, Ossola R, Aebersold R, Lee H, et al: Protein expression changes in ovarian cancer during the transition from benign to malignant. J Proteome Res 11: 2876-2889, 2012.
22. Gao Y, Meng H, Liu S, Hu J, Zhang Y, Jiao T, Liu Y, Ou J,
25. 
23. Silva JM, Bocek NJ, Berres MW, Ma X and Smith DR: LSINCT5
is over expressed in breast and ovarian cancer and affects
cellular proliferation. RNA Biol 8: 496-505, 2011.

24. Medrzycki M, Zhang Y, Zhang W, Cao K, Pan C, Laillier N,
McDonald JP, Bouhassira EE and Fan Y: Histone h1.3 suppresses
h19 noncoding RNA expression and cell growth of ovarian
cancer cells. Cancer Res 74: 6463-6473, 2014.

25. Guan Y, Kuo WL, Stilwell JL, Takano H, Lapuk AV, Fridlyand J,
Mao JH, Yu M, Miller MA, Santos JL, et al: Amplification of
PVT1 contributes to the pathophysiology of ovarian and breast
cancer. Clin Cancer Res 13: 5745-5755, 2007.

26. Khalil AM, Guttman M, Harte M, Garber M, Raj A,
Riveu Morales D, Thomas K, Presser A, Bernstein BE,
van Oudenaarden A, et al: Many human large intergenic
noncoding RNAs associate with chromatin-modifying complexes
and affect gene expression. Proc Natl Acad Sci USA 106:
11667-11672, 2009.

27. G Hendrickson D, Kelley DR, Tenen D, Bernstein B and Rinn JL:
Widespread RNA binding by chromatin-associated proteins.
Genome Biol 17: 28, 2016.

28. Salmena L, Poliseno L, Tay Y, Kats L and Pandolfi PP: A ceRNA
hypothesis: The Rosetta Stone of a hidden RNA language?
Cell 146: 353-358, 2011.

29. Zhou M, Wang X, Shi H, Cheng L, Wang Z, Zhao H, Yang L
and Sun J: Characterization of long non-coding RNA-associated
ceRNA network to reveal potential prognostic lncRNA biomarkers
in human ovarian cancer. Oncotarget 7: 12598-12611,
2016.

30. Cole SW, Nagaraja AS, Lutgendorf SK, Green PA and Sood AK:
Sympathetic nervous system regulation of the tumour
microenvironment. Nat Rev Cancer 15: 563-572, 2015.

31. Huan HB, Wen XD, Chen XJ, Wu L, Wu LL, Zhang YP, Zhang X,
Bie P, Qian C, et al: Sympathetic nervous system
promotes hepatocarcinogenesis by modulating inflammation
through activation of alpha1-adrenergic receptors of Kupffer
cells. Brain Behav Immun 59: 118-134, 2017.

32. Magnon C, Hall SJ, Lin J, Xue X, Gerber L, Freedland SJ and
Fremont PS: Autonomic nerve development contributes to
prostate cancer progression. Science 341: 1236361, 2013.

33. Luo M, Li Z, Wang W, Zeng Y, Liu Z and Qiu J: Long non-coding
RNA H19 increases bladder cancer metastasis by associating
with EZH2 and inhibiting E-cadherin expression. Cancer
Lett 333: 213-221, 2013.

34. Hirata H, Hinoda Y, Shahraray V, Deng G, Nakajima K,
Tabatabai ZL, Ishi N and Dahiya R: Long noncoding RNA
MALAT1 promotes aggressive renal cell carcinoma through
Ezh2 and interacts with miR-205. Cancer Res 75: 1322-1331,
2015.

35. Crea F, Wataghiki A, Quaglia L, Xue H, Pikor L, Polario A,
Wang Y, Lin D, Lam WL, Farrar WL, et al: Identification of
a long non-coding RNA as a novel biomarker and potential
therapeutic target for metastatic prostate cancer. Oncotarget 5:
764-774, 2014.

36. Zhou M, Zhong L, Xu W, Sun Y, Zhang Z, Zhao H, Yang L
and Sun J: Discovery of potential prognostic long non-coding RNA
biomarkers for predicting the risk of tumor recurrence of breast
cancer patients. Sci Rep 6: 31038, 2016.

37. Zhou M, Diao Z, Yue X, Chen Y, Zhao H, Cheng L and Sun J: Construction and analysis of dysregulated lncRNA-associated
cRNA network identified novel lncRNA biomarkers for early
diagnosis of human pancreatic cancer. Oncotarget 7:
56383-56394, 2016.

38. Zhou M, Guo M, He D, Wang X, Cui Y, Yang H, Hao D and
Sun J: A potential signature of eight long non-coding RNAs
predicts survival in patients with non-small cell lung cancer. J
Transl Med 13: 231, 2015.

39. Zhou M, Xu W, Yue X, Zhao H, Wang Z, Shi H, Cheng L and
Sun J: Relapse-related long non-coding RNA signature to improve
prognosis prediction of lung adenocarcinoma. Oncotarget 7:
29720-29738, 2016.

40. Ma Y, Lu Y and Lu B: MicroRNA and long non-coding RNA in
ovarian carcinoma: Translational insights and potential clinical
applications. Cancer Invest 34: 465-476, 2016.

41. Yim GW, Kim HJ, Kim JK, Kim SW, Kim S, Nam EJ and
Kim YT: Long non-coding RNA HOXA11 antisense promotes
cell proliferation and invasion and predicts patient prognosis in
serous ovarian cancer. Cancer Res Treat: Oct 11, 2016 (Epub
ahead of print). doi: 10.4143/crt.2016.263.