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Original Article

LncRNA CCND2-AS1 is up-regulated and regulates proliferation, migration, and invasion in breast cancer

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Abstract: Many long non-coding RNAs (lncRNAs) have been found to exert influences on biological processes including tumorigenesis. Many lncRNAs have been reported as potential therapeutic targets and prognostic biomarkers in multiple cancers. CCND2 antisense RNA 1 (CCND2-AS1) is an lncRNA recently reported to be involved in the progression of glioma cancers. However, whether CCND2-AS1 is associated with progression of breast cancer remains unknown. In this study, quantitative real-time polymerase chain reaction (qRT-PCR) was used to measure CCND2-AS1 gene expression in breast cancer cell in lines. CCND2-AS1 expression was significantly over-expressed compared to normal breast epithelial cells. Gain-and loss-of-function experiments were performed *in vitro* to investigate the role of CCND2-AS1, where we found that CCND2-AS1 knockdown in MDA-MB-231 significantly suppressed cell proliferation, migration, and invasion. In contrast, CCND2-AS1 overexpression in BT-549 had the opposite effects. Our findings indicate that lncRNA CCND2-AS1 is a gene associated with breast cancer and might become a potential therapeutic target.

Keywords: LncRNA, CCND2-AS1, breast cancer

Introduction

Breast cancer is one of the most common diseases in women. With development of medical technology, breast cancer has been treated by means of surgery, chemotherapy, endocrine, or targeted therapies but it still has a high death rate in American women [1, 2]. Therefore, it is urgent for us to explore the process of breast cancer and find new biomarkers at an early stage.

Long non-coding RNAs (lncRNAs) are a class of non-protein-coding transcripts that are longer than 200 nucleotides [3, 4]. LncRNAs used to be considered useless, but recent research demonstrates that dysregulation of lncRNAs may contribute to the cancer progression. Aberrant lncRNAs can drive cancer through the obstruction of proliferation, apoptosis, cell migration and invasion [5-7]. In breast cancer, many lncRNAs have been demonstrated to directly influence cancer developmental process [8-10]. Genxiang Liu et al. found that long non-coding RNA OR3A4 is overexpression in BC tissues and cell lines, and that high expression OR3A4 can promote proliferation and migration in breast cancer [11]. Li et al. found that lncNEAT1 facilitates cell growth and invasion via the miR-211/HMGA2 axis in breast cancer [12].

CCND2-AS1 is the lncRNA transcribed from the antisense strand of CCND2, a transcript of 553 bp. CCND2 antisense RNA 1 (CCND2-AS1) was first found in glioma. Hua Zhang et al. demonstrated that CCND2-AS1 is upregulated in glioma cell lines and highly expression CCND2-AS1 could promote the proliferation of glioma cell through Wnt/β-catenin signaling [13]. However, the underlying function of CCND2-AS1 in breast cancer is not exactly clear. In this study, we examined the expression CCND2-AS1 in breast cancer by using qRT-PCR. Our study also demonstrated the function of ccnd2-as1 in breast cancer cell lines through gain-and loss-of-function experiments. These results imply that

CCND2-AS1 is a gene associated with breast cancer and it might become a therapeutic target.

Materials and methods

Cell cultures and growth conditions

MDA-MB-231, BT-549, MCF-7, T-47D and MCF-10A cells were used in this study. These cells were obtained from Shanghai Cell Biology, Institute of the Chinese Academy of Sciences (Shanghai, China). MDA-MB-231, MCF-7, and T-47D were cultured in Dulbecco's Modified Eagle's Medium DMEM (Gibco, Grand Island, NY, USA) supplemented with 10% FBS (Gibco, Grand Island, NY, USA). BT-549 were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% FBS (Gibco, Grand Island, NY, USA). MCF-10A cells were cultured in DMEM-F12 (Gibco, Grand Island, NY, USA) supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin, 2 mm of L-glutamine, 20 ng/ml of epidermal growth factor and 10% FBS (Gibco, Grand Island, NY, USA). These cells were incubated in a standard cell culture incubator (Thermo, Waltham, MA, USA) at 37°C with 5% CO_2.

Cell transfection

MDA-MB-231 was transfected using Lipofectamine RNAiMAX transfection reagent (Invitrogen) by following the manufacturer's protocol. Cells were plated at 100,000 per plate one day before transfection. CCND2-AS1 was silenced using 10 nM siRNA for 48 h. The siRNA sequences used in the study are: CCND2-AS1 siRNAs target the following sequences: CCND2-AS1 siRNA-1, Forward 5'-GGGCUGGUCUCUUUGAGUUTT-3' and Reverse 5'-AACUCAAAGAGACCAGCCCTT-3'; CCND2-AS1 siRNA-2, Forward 5'-CCAACAGACUCCUCCUGAUTT-3' and Reverse 5'-AUCAGGAGGAGUCUGUUGGTT-3'; CCND2-AS1 siRNA-3, Forward 5'-GCCAGAUACUCUGUUGCCATT-3' and Reverse 5'-UGGCAACAGAGUAUCUGGCTT-3'. Both siRNAs were provided by GenePharma. To overexpress CCND2-AS1, vectors which containing the full length of CCND2-AS1 were constructed into CMV-MCS-EGFP-SV40-Neomycin.

RNA extraction and quantitative real-time PCR

Total RNA was isolated using TRIzol reagent according to the manufacturer's instructions (Invitrogen, USA). RNA samples were stored at -80°C. The purity of the isolated RNA was measured at 260/280 nm by spectrophotometry (Thermo, San Jose, CA, USA). Real-time reactions were run and analyzed by using a real-time PCR system (Applied Biosystems 7500). The relative expression of mRNA was calculated using the comparative cycle threshold (CT) ($2^{-\Delta\Delta CT}$) method with GAPDH as the endogenous control to normalize the data. The sequences of the primers used were: Ccnd2-as1 forward: 5'-TCTGGTATCTGGCGTTCT-3' and Reverse: 5'-GCTTGTCTGAGGAATGTTG-3'; GADPH Forward: 5'-GTCTCCTCTGACTTCAACAGCG-3' and Reverse: 5'-ACCACCCTGTTGCTGTAGCCAA-3'.

Colony forming assay

Using trypsin digest cells and exposing cells to the indicated treatments accordingly. Cells were then seeded into 6-well plates (1200 cells/wells). After culture for 10 days, colonies were fixed by 4% paraformaldehyde and stained by crystal violet dye and these images were captured by camera. Experiments were performed in triplicate.

CCK-8 proliferation assay

According to the manufacturer's protocol, CCK-8 assay was used to assess cell proliferation. Approximately 1500 cells were plated into 96-well plates. After cells were adhered, 10 μl of CCK-8 solution was added to each well and incubated at 37°C for 2 h. Cell proliferation curves were drawn by measuring 450 nm absorbance at the indicated time point. Experiments were performed in triplicate.

Invasion and migration assay

For transwell assays, cells were trypsinized by Trypsin and collected with medium containing 10% FBS. Invasion of cells was measured in Matrigel (BD, Franklin Lakes, NJ, USA) -coated transwell inserts (6.5 mm, Costar, Manassas, VA, USA) containing polycarbonate filters with 8-μm pores. The inserts were coated with 50 μl of 1 mg/ml Matrigel matrix according to the manufacturer's recommendations. A total of 30,000 cells (~250 μl) were transferred into the upper chamber. The lower chamber was filled with 600 μl medium containing 20% FBS. The plate was then put into the incubator. After 24 h, the membrane was fixed with 4% paraformaldehyde and stained with 0.4% crystal violet

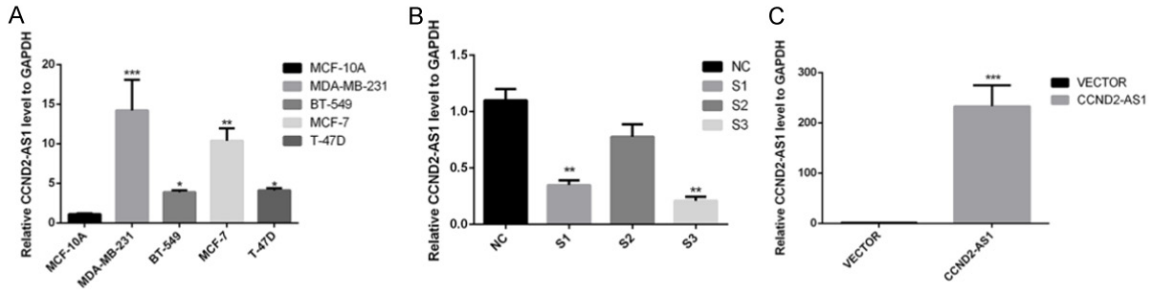

Figure 1. CCND2-AS1 expression in breast cancer cells at the transcriptional level. A. The relative expression of CCND2-AS1 gene (compared with the GAPDH gene) using qRT-PCR. Compared to the other cell lines, MDA-MB-231 cells exhibited the highest CCND2-AS1 expression while BT-549 cells exhibited the lowest expression. B. The relative expression of CCND2-AS1 gene (compared with the GAPDH gene) in four groups. Compared with corresponding control group, the expression of CCND2-AS1 gene in S1 and S3 group was lower. C. The relative expression of CCND2-AS1 gene (compared with the GAPDH gene) in two groups. Compared with the vector group, the expression of CCND2-AS1 gene in the CCND2-AS1 group was higher. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ in comparison with the control group using Student's t-test. A logarithmic scale of $2^{-\Delta\Delta Ct}$ is used to represent the fold change in quantitative real-time PCR detection.

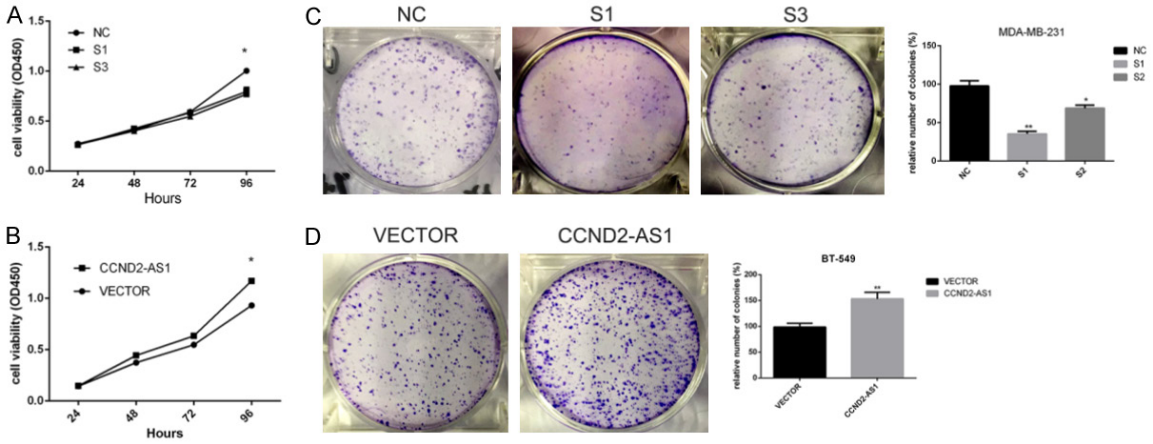

Figure 2. Dysregulation of lnc CCND2-AS1 gene expression in breast cancer cells inhibits proliferation. A, C. Cck-8 assays and colony formation assays in down-regulation MDA-MB-231 cells and their corresponding control cells. B, D. Cck-8 assays and colony formation assays in up-regulation BT-549 cells and their corresponding control cells. $*P < 0.05$ in comparison with the NC or vector group using Student's t-test.

solution for 20 min. Motility assays were similar to Matrigel invasion assay except that the transwell insert was not coated with Matrigel. Cell migration and invasion ability were assessed by counting the cells that had migrated through the membrane. Five random fields of view were selected and images captured under a microscope at a magnification of ×20.

Statistical analysis

Data are presented as mean ± SE. All statistical analyses were performed using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). Differences were considered to be statistically significant at $P < 0.05$. Student's t-test (two-tailed) was performed to analyze differences between groups.

Results

LncRNA CCND2-AS1 is upregulated in breast cancer cell lines

A recent study has indicated that lncRNA CCND2-AS1 is overexpressed in glioma cancer and is critical in tumor progression. To confirm the role of CCND2-AS1 in breast cancer, we first assessed CCND2-AS1 expression level in several breast cancer cell lines and a normal

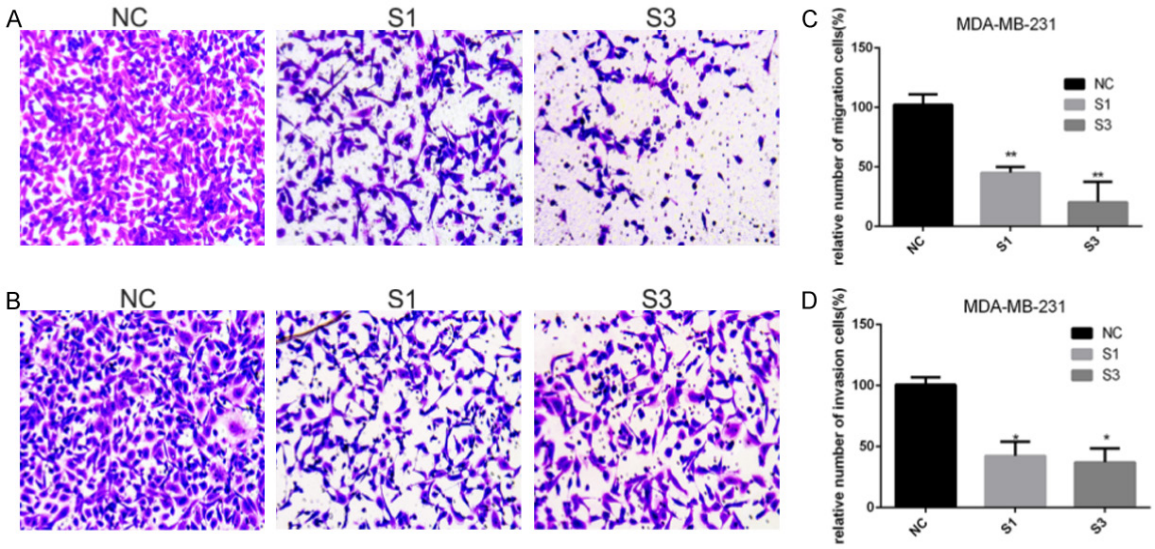

Figure 3. Down-regulation of CCND2-AS1 gene expression in MDA-MB-231 cell inhibiting migration and invasion. A, B. Transwell migration and invasion assays in down-regulation lncRNA CCND2-AS1 cells and their corresponding control cells. C, D. Quantitative results of migration and invasion assays. The stained cells were manually counted from 5 randomly selected fields and normalized with cell proliferation. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 in comparison with the NC group using Student's t-test.

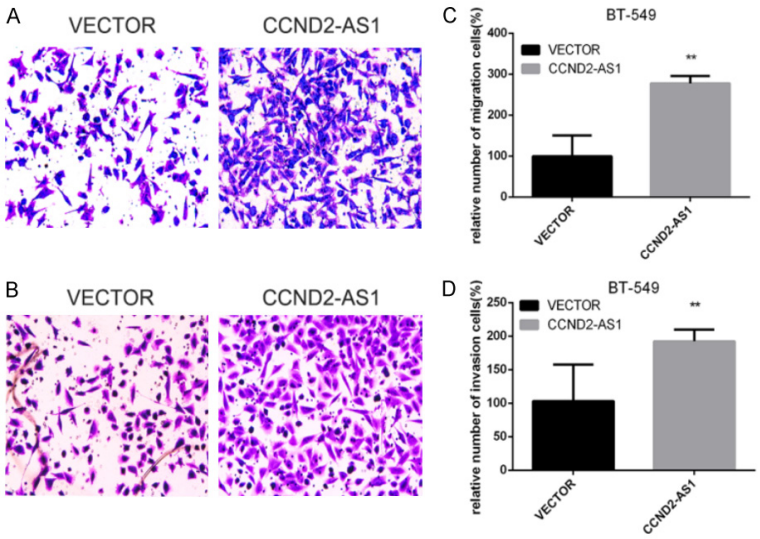

Figure 4. Up-regulation CCND2-AS1 gene expression in BT-549 cells by inhibiting migration and invasion. A, B. Transwell migration and invasion assays in up-regulation lncRNA CCND2-AS1 cells and their corresponding control cells. C, D. Quantitative results of migration and invasion assays. The stained cells were manually counted from 5 randomly selected fields and normalized with cell proliferation. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 in comparison with the vector group using Student's t-test.

breast cell line by qRT-PCR. We found that CCND2-AS1 was expressed a higher level in breast cancer cell lines (MDA-MB-231, BT-549, MCF-7, and T-47D) than in normal breast cell line MCF-10A (**Figure 1A**). It suggests that lncRNA CCND2-AS1 was upregulated in breast cancer.

LncRNA CCND2-AS1 regulates breast cancer lines proliferation

In our experience, MDA-MB-231 was the highest expression level of lncRNA CCND2-AS1 between the breast cancer cells, while BT-549 was the lowest expression level of lncRNA CCND2-AS1. So we selected MDA-MB-231 and BT-549 as the research cell lines. To further examine whether lncRNA CCND2-AS1 functions in breast cancer progression, we knocked down lncRNA CCND2-AS1 expression in MDA-MB-231 using small interfering RNA and overexpressed the lncRNA CCND2-AS1 using a pcDNA (**Figure 1B** and **1C**). Then we performed colony formation assays and cell proliferation assays. We found that down-regulated lncRNA CCND2-AS1 effectively inhibited breast cancer cell line proliferation (**Figure 2A**) and colony formation (**Figure 2C**) compared with the control group

while upregulation of lncRNA CCND2-AS1 enhanced breast cancer cell line proliferation (**Figure 2B**) and colony formation (**Figure 2D**).

LncRNA CCND2-AS1 regulates breast cancer lines migration and invasion

Cell motility and invasiveness ability closely correlate with tumor progression. Our experience found that down-regulated lncRNA CCND2-AS1 significantly inhibited breast cancer cell lines migration capacity compared with the control groups and upregulated lncRNA CCND2-AS1 has the opposite result (**Figures 3A** and **4A**). Invasion assays also showed that down-regulated lncRNA CCND2-AS1 effectively inhibited invasion capacity in breast cancer cell lines (**Figure 3B**) while upregulated lncRNA CCND2-AS1 enhanced invasion capacity (**Figure 4B**).

Discussion

Breast cancer is the most common malignant tumor in the world with variety forms of treatment [1, 14]. With improvements of medical care, early recognition of breast cancer is more common. We can determine the subtype based on pathological results. However, breast cancer is also a heterogeneous disease. Therefore, finding novel molecular biomarkers which can predict the process of breast cancer is very urgent.

LncRNAs exert much influence in different manners, including the epigenetic and regulations posttranscriptional regulation of RNA splicing [15, 16]. As so far, many studies have demonstrated that lncRNAs promote tumorigenesis. For instance, lncRNA may competitively combine with miRNA and act as a sponge, which induces miRNA disability and promotes cancer progression. Lu et al. found that BC032469 can function as a ceRNA to impair miR-1207-5p-dependent hTERT down-regulation in gastric cancer [17]. Long non-coding RNA Loc554202 induces caspase cleavage cascades that lead to apoptosis in colorectal cancer cells [18]. J Huang et al. found that long non-coding RNA UCA1 can supper p27 led to promote breast tumor growth [19]. Long non-coding RNA MEG3 promotes the proliferation of glioma cells by targeting the Wnt/β-catenin signal pathway [20].

H. Zhang first found the lncRNA CCND2-AS1 in glioma cell. They found that highly expressed lncRNA CCND2-AS1 promotes glioma cell proliferation through Wnt/b-catenin signaling [13]. However, there have been no articles describing the role of lncRNA CCND2-AS1 in breast cancer.

In this study, we found the lncRNA CCND2-AS1 is overexpressed in breast cancer cell lines. We demonstrated that down-regulation of lncRNA CCND2-AS1 inhibits proliferation, migration, and invasion in MDA-MB-231 while upregulated lncRNA CCND2-AS1 enhances proliferation, migration, and invasion of BT-549. These findings imply that highly expressed lncRNA CCND2-AS1 promotes breast cell proliferation, migration, and invasion.

However, our study still has several limitations. First, *in vivo* experiments needed to be detected to better validate the biological effects of CCND2-AS1. Second, previous studies have found that perturbation of antisense RNA may influence the expression of the sense gene [21, 22]. Cyclin D2 is a member of the D-type cyclin proteins family that are periodically expressed in the cell cycle [23]. Therefore, the mechanism of the interplay between CCND2-AS1 and cyclin D2(CCND2) still remains unknown.

Taken together, in our study we demonstrated that the lncRNA CCND2-AS1 is commonly upregulated in breast cancer cells. Over-expressed lncRNA CCND2-AS1 promotes breast cancer cell proliferation, migration, and invasion while knockdown lncRNA CCND2-AS1 is inhibitory. These results suggest that lncRNA CCND2-AS1 plays an important role in breast tissue and it might become a potential therapeutic target.

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Disclosure of conflict of interest

None.

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References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Pelkonen M, Luostari K, Tengström M, Ahonen H, Berdel B, Kataja V, Soini Y, Kosma VM, Mannermaa A. Low expression levels of hepsin and TMPRSS3 are associated with poor breast cancer survival. BMC Cancer 2015; 15: 431.

[3] Zhao W, Luo J, Jiao S. Comprehensive characterization of cancer subtype associated long non-coding RNAs and their clinical implications. Sci Rep 2014; 4: 6591.

[4] Consortium EP, Birney E, Stamatoyannopoulos JA, Dutta A, Guigo R, Gingeras TR, Margulies EH, Weng Z, Snyder M, Dermitzakis ET, Thurman RE, Kuehn MS, Taylor CM, Neph S, Koch CM, Asthana S, Malhotra A, Adzhubei I, Greenbaum JA, Andrews RM, Flicek P, Boyle PJ, Cao H, Carter NP, Clelland GK, Davis S, Day N, Dhami P, Dillon SC, Dorschner MO, Fiegler H, Giresi PG, Goldy J, Hawrylycz M, Haydock A, Humbert R, James KD, Johnson BE, Johnson EM, Frum TT, Rosenzweig ER, Karnani N, Lee K, Lefebvre GC, Navas PA, Neri F, Parker SC, Sabo PJ, Sandstrom R, Shafer A, Vetrie D, Weaver M, Wilcox S, Yu M, Collins FS, Dekker J, Lieb JD, Tullius TD, Crawford GE, Sunyaev S, Noble WS, Dunham I, Denoeud F, Reymond A, Kapranov P, Rozowsky J, Zheng D, Castelo R, Frankish A, Harrow J, Ghosh S, Sandelin A, Hofacker IL, Baertsch R, Keefe D, Dike S, Cheng J, Hirsch HA, Sekinger EA, Lagarde J, Abril JF, Shahab A, Flamm C, Fried C, Hackermüller J, Hertel J, Lindemeyer M, Missal K, Tanzer A, Washietl S, Korbel J, Emanuelsson O, Pedersen JS, Holroyd N, Taylor R, Swarbreck D, Matthews N, Dickson MC, Thomas DJ, Weirauch MT, Gilbert J, Drenkow J, Bell I, Zhao X, Srinivasan KG, Sung WK, Ooi HS, Chiu KP, Foissac S, Alioto T, Brent M, Pachter L, Tress ML, Valencia A, Choo SW, Choo CY, Ucla C, Manzano C, Wyss C, Cheung E, Clark TG, Brown JB, Ganesh M, Patel S, Tammana H, Chrast J, Henrichsen CN, Kai C, Kawai J, Nagalakshmi U, Wu J, Lian Z, Lian J, Newburger P, Zhang X, Bickel P, Mattick JS, Carninci P, Hayashizaki Y, Weissman S, Hubbard T, Myers RM, Rogers J, Stadler PF, Lowe TM, Wei CL, Ruan Y, Struhl K, Gerstein M, Antonarakis SE, Fu Y, Green ED, Karaöz U, Siepel A, Taylor J, Liefer LA, Wetterstrand KA, Good PJ, Feingold EA, Guyer MS, Cooper GM, Asimenos G, Dewey CN, Hou M, Nikolaev S, Montoya-Burgos JI, Löytynoja A, Whelan S, Pardi F, Massingham T, Huang H, Zhang NR, Holmes I, Mullikin JC, Ureta-Vidal A, Paten B, Seringhaus M, Church D, Rosenbloom K, Kent WJ, Stone EA; NISC Comparative Sequencing Program; Baylor College of Medicine Human Genome Sequencing Center; Washington University Genome Sequencing Center; Broad Institute; Children's Hospital Oakland Research Institute, Batzoglou S, Goldman N, Hardison RC, Haussler D, Miller W, Sidow A, Trinklein ND, Zhang ZD, Barrera L, Stuart R, King DC, Ameur A, Enroth S, Bieda MC, Kim J, Bhinge AA, Jiang N, Liu J, Yao F, Vega VB, Lee CW, Ng P, Shahab A, Yang A, Moqtaderi Z, Zhu Z, Xu X, Squazzo S, Oberley MJ, Inman D, Singer MA, Richmond TA, Munn KJ, Rada-Iglesias A, Wallerman O, Komorowski J, Fowler JC, Couttet P, Bruce AW, Dovey OM, Ellis PD, Langford CF, Nix DA, Euskirchen G, Hartman S, Urban AE, Kraus P, Van Calcar S, Heintzman N, Kim TH, Wang K, Qu C, Hon G, Luna R, Glass CK, Rosenfeld MG, Aldred SF, Cooper SJ, Halees A, Lin JM, Shulha HP, Zhang X, Xu M, Haidar JN, Yu Y, Ruan Y, Iyer VR, Green RD, Wadelius C, Farnham PJ, Ren B, Harte RA, Hinrichs AS, Trumbower H, Clawson H, Hillman-Jackson J, Zweig AS, Smith K, Thakkapallayil A, Barber G, Kuhn RM, Karolchik D, Armengol L, Bird CP, de Bakker PI, Kern AD, Lopez-Bigas N, Martin JD, Stranger BE, Woodroffe A, Davydov E, Dimas A, Eyras E, Hallgrímsdóttir IB, Huppert J, Zody MC, Abecasis GR, Estivill X, Bouffard GG, Guan X, Hansen NF, Idol JR, Maduro VV, Maskeri B, McDowell JC, Park M, Thomas PJ, Young AC, Blakesley RW, Muzny DM, Sodergren E, Wheeler DA, Worley KC, Jiang H, Weinstock GM, Gibbs RA, Graves T, Fulton R, Mardis ER, Wilson RK, Clamp M, Cuff J, Gnerre S, Jaffe DB, Chang JL, Lindblad-Toh K, Lander ES, Koriabine M, Nefedov M, Osoegawa K, Yoshinaga Y, Zhu B, de Jong PJ. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 2007; 447: 799-816.

[5] Bergmann JH, Spector DL. Long non-coding RNAs: modulators of nuclear structure and function. Curr Opin Cell Biol 2014; 26: 10-18.

[6] George J, Patel T. Noncoding RNA as therapeutic targets for hepatocellular carcinoma. Semin Liver Dis 2015; 35: 63-74.

[7] Yan TH, Lu SW, Huang YQ, Que GB, Chen JH, Chen YP, Zhang HB, Liang XL, Jiang JH. Upregulation of the long noncoding RNA HOTAIR predicts recurrence in stage Ta/T1 bladder cancer. Tumour Biol 2014; 35: 10249-10257.

[8] Huang J, Zhang A, Ho TT, Zhang Z, Zhou N, Ding X, Zhang X, Xu M, Mo YY. Linc-RoR promotes c-Myc expression through hnRNP I and AUF1. Nucleic Acids Res 2016; 44: 3059-3069.

[9] Chen S, Wang Y, Zhang JH, Xia QJ, Sun Q, Li ZK, Zhang JG, Tang MS, Dong MS. Long non-coding

RNA PTENP1 inhibits proliferation and migration of breast cancer cells via AKT and MAPK signaling pathways. Oncol Lett 2017; 14: 4659-4662.
[10] Sun L, Li Y, Yang B. Downregulated long noncoding RNA MEG3 in breast cancer regulates proliferation, migration and invasion by depending on p53's transcriptional activity. Biochem Biophys Res Commun 2016; 478: 323-329.
[11] Liu G, Hu X, Zhou G. Long non-coding RNA OR3A4 promotes proliferation and migration in breast cancer. Biomed Pharmacother 2017; 96: 426-433.
[12] Li X, Wang S, Li Z, Long X, Guo Z, Zhang G, Zu J, Chen Y, Wen L. The lncRNA NEAT1 facilitates cell growth and invasion via the miR-211/HMGA2 axis in breast cancer. Int J Biol Macromol 2017; 105: 346-353.
[13] Zhang H, Wei DL, Wan L, Yan SF, Sun YH. Highly expressed lncRNA CCND2-AS1 promotes glioma cell proliferation through Wnt/beta-catenin signaling. Biochem Biophys Res Commun 2017; 482: 1219-1225.
[14] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.
[15] Mercer TR, Mattick JS. Structure and function of long noncoding RNAs in epigenetic regulation. Nat Struct Mol Biol 2013; 20: 300-307.
[16] Dinger ME, Gascoigne DK, Mattick JS. The evolution of RNAs with multiple functions. Biochimie 2011; 93: 2013-2018.
[17] Lu MH, Tang B, Zeng S, Hu CJ, Xie R, Wu YY, Wang SM, He FT, Yang SM. Long noncoding RNA BC032469, a novel competing endogenous RNA, upregulates hTERT expression by sponging miR-1207-5p and promotes proliferation in gastric cancer. Oncogene 2016; 35: 3524-3534.
[18] Ding J, Lu B, Wang J, Wang J, Shi Y, Lian Y, Zhu Y, Wang J, Fan Y, Wang Z, De W, Wang K. Long non-coding RNA Loc554202 induces apoptosis in colorectal cancer cells via the caspase cleavage cascades. J Exp Clin Cancer Res 2015; 34: 100.
[19] Huang J, Zhou N, Watabe K, Lu Z, Wu F, Xu M, Mo YY. Long non-coding RNA UCA1 promotes breast tumor growth by suppression of p27 (Kip1). Cell Death Dis 2014; 5: e1008.
[20] Gong X, Huang M. Long non-coding RNA MEG3 promotes the proliferation of glioma cells through targeting Wnt/β-catenin signal pathway. Cancer Gene Therapy 2017; 24: 381-385.
[21] Rosok O, Sioud M. Systematic identification of sense-antisense transcripts in mammalian cells. Nat Biotechnol 2004; 22: 104-108.
[22] Katayama S, Tomaru Y, Kasukawa T, Waki K, Nakanishi M, Nakamura M, Nishida H, Yap CC, Suzuki M, Kawai J, Suzuki H, Carninci P, Hayashizaki Y, Wells C, Frith M, Ravasi T, Pang KC, Hallinan J, Mattick J, Hume DA, Lipovich L, Batalov S, Engström PG, Mizuno Y, Faghihi MA, Sandelin A, Chalk AM, Mottagui-Tabar S, Liang Z, Lenhard B, Wahlestedt C; RIKEN Genome Exploration Research Group; Genome Science Group (Genome Network Project Core Group); FANTOM Consortium. Antisense transcription in the mammalian transcriptome. Science 2005; 309: 1564-1566.
[23] Chiles TC. Regulation and function of cyclin D2 in B lymphocyte subsets. J Immunol 2004; 173: 2901-2907.