Genome-wide study of ER-regulated lncRNAs shows AP000439.3 may function as a key regulator of cell cycle in breast cancer

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Received February 18, 2017; Accepted July 12, 2017

DOI: 10.3892/or.2017.5975

Abstract. Estrogen receptor (ER) plays important roles in cell growth, development and tumorigenesis. Although ER-regulated genes have been extensively investigated, little is known about roles of ER-regulated lncRNAs in breast cancer. Here, we conducted genome-wide study of ER-regulated lncRNAs by using RNA-seq, ChIP-seq and TCGA data. A total of identified 114 ER-regulated lncRNAs were identified, many of them were overexpressed in ER$^+$ breast cancer and co-expressed with some key regulators. Silencing one of most prominent lncRNA, AP000439.3, resulted in inhibition of cell cycle progression and proliferation. Further study revealed AP000439.3 can regulate expression of CCND1 through enhancing estrogen receptor induction of CCND1. This finding revealed lncRNAs may serve as important effectors of ER in regulation of gene expression and cell phenotype in breast cancer.

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Abbreviations: ER, estrogen receptor; lncRNA, long non-coding RNA; EREs, estrogen response elements; lincRNA, long intergenic non-coding RNA; DE, differential expression; qPCR, quantitative polymerase chain reaction; TCGA, The Cancer Genome Atlas; RPM, reads per million; FPKM, reads per kilobase of exon model per million mapped reads; ChIP, chromatin immunoprecipitation

Key words: estrogen receptor, lncRNA, breast cancer, CCND1, AP000439.3

Introduction

Estrogen plays critical roles in normal mammary gland development, physiological processes as well as tumorigenesis. Estrogen receptor (ER) is the major mediator of the effect of estrogen. After binding to estrogen, ER is activated and translocates into the cell nucleus (1). In the nucleus, ER can transcriptionally regulate target genes either by directly binding to estrogen response elements (EREs) or through protein-protein interactions with other transcriptional factors such as AP1, SP1 and NF-κB (2). Moreover, ~70% of breast cancers express ER. ER is essential for the initiation and development of breast cancers (1,3,4). The pro-oncogenic effect of ER is mediated primarily by transcriptionally regulating its target genes. Various genes that play crucial roles in cell proliferation and apoptosis such as *c-Myc*, *CCND1* and *BCL2* have been identified as ER-regulated genes. Hundreds of protein-coding genes have been identified as ER-regulated genes by using microarray and RNA-seq as well as ChIP-seq (5-9).

Long non-coding RNAs (lncRNAs) refer to non-coding RNAs consisting of >200 nucleotides. Recently, the important roles of lncRNAs in breast cancer cell proliferation, apoptosis, metastasis and endocrine resistance have been reported (10-12). Because of their crucial roles in breast cancer, we explored ER-regulated lncRNAs. Although a few studies have been carried out to identify ER-regulated lncRNAs very recently (13-15), their roles in regulation of gene expression and cell phenotype remains largely unknown. Here, we report a genome-wide study of ER-regulated lncRNAs by conducting an integrate analysis of ChIP-seq, strand-specific RNA-seq and TCGA clinical data. We observed that many of these ER-regulated lncRNAs were overexpressed in ER$^+$

breast cancer and exhibited co-expression with several key regulator proteins. Moreover, we found one of most prominent lncRNAs, *AP000439.3*, can promote cell cycle progression through enhancing *CCDN1* expression induced by estrogen. These findings reveal ER can regulate many lncRNAs that exhibit important functions in regulation of gene expression and cell phenotype in breast cancer.

Materials and methods

Datasets and computational analysis. The 17β-estradiol (E2) and vehicle treated MCF7 RNA-seq data (pair-end, strand-specific) was previously reported by Dago *et al* (16) and obtained from GEO dataset (GSE64590). For RNA-seq analysis, the sequenced reads were aligned to human reference genome (Hg38) and transcriptome (GENCODE.v23) using STAR (17) and then processed by RSEM (18). Genes with FDR<0.05 generated by both DESeq (19) and edgeR (20) were considered as differentially expressed genes.

ERα ChIP-seq of estrogen treated and untreated MCF7 were reported by Franco *et al* (21) and obtained from GEO dataset (GSE59530). Reads of ChIP-seq were aligned to human reference genome (Hg38) using BOWTIE (22). The estrogen treated peaks were generated by MACS (23) using untreated as control. The ER binding sites within ±100 kb region of transcriptional start sites (TSS) of protein-coding genes and lncRNAs (GENCODE.v23) were considered as potential ER regulatory sites.

The TCGA lncRNA data were downloaded from TANRIC database (24), mRNA and RPPAs data were downloaded from Broad Dashboard-Stddata (https://confluence.broadinstitute.org/display/GDAC/Dashboard-Stddata).

Cell culture and treatments. MCF7, ZR-75-1 and T47D cells were obtained from ATCC. MCF7 cells were cultured in Eagle's minimum essential medium supplemented with 10% FBS; ZR-75-1 and T47D were cultured in RPMI-1640 media supplemented with 10% FBS. Before estrogen treatments, the cells were grown for 72 h in phenol red-free MEM Eagle medium supplemented with 10% charcoal-dextran-treated FBS. The cells were then treated with ethanol (vehicle) or 10 nM E2 for 24 h. siRNAs were synthesized in GenePharma Co. (Shanghai, China). The sequences of siRNA are listed in Table II. siRNA was transfected at a final concentration of 50 nM using Lipofectamine 2000 reagent (Invitrogen).

Real-time quantitative PCR and western blotting. Cells were grown and treated as described above and then RNA was collected using TRIzol reagent (Invitrogen), RNA was reverse-transcribed using PrimeScript RT reagent kit (Takara RR047A) and analyzed by LightCycler® 96 real-time PCR thermocycler (Roche), primers are listed in Table II. The lncRNA expression was normalized to β-actin transcript as an internal standard. The CCND1 and α-tubulin protein were detected by western blotting using cyclin D1 mouse mAb (BD Pharmigen #556470) and α-tubulin antibody (Santa Cruz, SC-5286).

Cell cycle analysis and colony formation assay. Seventy-two hours after siRNA transfection, cells were digested and washed with PBS and then fixed in 70% ethanol overnight at 4˚C, then incubated with RNase and propidium iodide (PI) for 10 min and analyzed by flow cytometry.

For colony forming analysis, 1,000 cells were plated in 6-well plates and grown for 2 weeks, colonies were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The number of colonies were counted and analyzed by ImageJ software.

Chromatin immunoprecipitation (ChIP) assay. The ChIP assay were performed as previously described (25) with MCF7 cells using 3 μg normal IgG (CST, #2729) and ER (Santa Cruz sc-543) antibody. The ChIP PCR primers were listed in Table II.

Statistical analysis. Wilcoxon rank-sum test was used for comparing fold change between lncRNA and protein. Spearman's rank moment correlation coefficient was calculated for analysis of the co-expression of lncRNAs and RPPAs, Pearson's product moment correlation for lncRNA-mRNA co-expression. For qRT-PCR and colony formation assay, Student's t-test was used to test for statistical significance of the differences between the different group parameters. p-values of <0.05 was considered statistically significant.

Results

Global identification and characterization of ER-regulated lncRNAs. To identify ER-regulated lncRNAs in a genome-wide manner, we exploited published RNA-seq of E2- and vehicle-treated cells that were originally used for analyzing alternative mRNA splicing (16) (GSE64590). We chose this dataset because: i) this RNA-seq was strand-specific, which was superb for analyzing antisense transcripts that overlapped with host genes. ii) Three repetitions with high reproducibility occurred in this dataset. We conducted an analysis of differential expression (DE) transcripts using edgeR (20) and DEseq (19) processed by RSEM (18). We observed 2,869 DE transcripts, which included 1,784 upregulated and 1,085 downregulated transcripts. The dominant types of DE transcripts were protein-coding genes (92.5%); 147 lncRNAs were found to express differentially upon E2 treatment (Fig. 1A). Of these lncRNAs, 45.6% were long intergenic RNA (lincRNAs), 19.7% were antisense and 16.3% were processed transcripts (Fig. 1B). DE lncRNAs showed significantly lower expression than protein-coding genes (Fig. 1C), which is in agreement with previous reports (26). Despite low expression of DE lncRNAs, we found DE lncRNAs exhibited a higher fold-change than DE protein-coding genes in the presence of estrogen (Fig. 1D). LncRNAs tend to be more specifically expressed than protein (27,28); moreover, lncRNAs differ not just between tissues, but also between closely related cell types, which indicates lncRNAs are likely under stricter regulations (29). In agreement with this notion, our analysis revealed lncRNAs were under more rigorous regulation of activated ER. To investigate whether these DE transcripts were transcriptionally regulated by ER, we conducted an analysis of ER binding sites using published ChIP-seq data, and 114 of 147 (77.6%) DE lncRNAs had at least one ER binding site within their genomic domain (within ± 100 kb from TSS). Half of these lncRNAs were upregulated by E2 and these lncRNAs were

Table I. List of ER-upregluated lncRNAs.

Ensembl ID	LncRNA name	Fold change (log2)	FDR
ENSG00000273565.1	CTD-3075F15.1	5.13	0.000258667
ENSG00000249346.6	LINC01016	4.30	1.89E-107
ENSG00000266036.1	RP11-452I5.2	4.04	3.29E-06
ENSG00000254290.1	RP11-150O12.3	2.74	6.46E-08
ENSG00000258354.1	MIR3180-1	2.69	6.93E-06
ENSG00000272472.1	RP11-95G17.2	2.51	5.95E-15
ENSG00000244265.1	SIAH2-AS1	2.48	0.000112224
ENSG00000227036.6	LINC00511	1.97	5.34E-30
ENSG00000259459.5	RP11-321G12.1	1.86	0.000182177
ENSG00000229525.1	AC053503.4	1.83	3.17E-06
ENSG00000233885.7	YEATS2-AS1	1.48	2.03E-14
ENSG00000259080.1	RP11-158I13.2	1.46	1.04E-05
ENSG00000261578.1	RP11-21L23.2	1.39	1.73E-26
ENSG00000280186.1	RP11-483I13.6	1.35	9.59E-53
ENSG00000204792.2	LINC01291	1.32	1.38E-21
ENSG00000253125.1	RP11-459E5.1	1.24	6.02E-05
ENSG00000260401.1	RP11-800A3.4	1.23	2.02E-94
ENSG00000261051.1	RP11-274H2.5	1.22	1.07E-16
ENSG00000266709.1	RP11-214O1.2	1.19	1.26E-05
ENSG00000223749.7	MIR503HG	1.18	2.99E-42
ENSG00000280924.1	LINC00628	1.16	2.97E-05
ENSG00000226471.6	CTA-292E10.6	1.16	9.45E-19
ENSG00000277159.1	RP11-88E10.4	1.12	2.90E-09
ENSG00000281207.1	SLFNL1-AS1	1.03	6.93E-06
ENSG00000280073.1	CTD-2525P14.5	0.95	1.47E-05
ENSG00000232956.8	SNHG15	0.93	1.38E-37
ENSG00000230836.1	LINC01293	0.84	5.77E-15
ENSG00000246174.7	KCTD21-AS1	0.79	3.09E-08
ENSG00000236144.6	TMEM147-AS1	0.42	8.75E-07
ENSG00000213062.4	RP1-206D15.6	0.75	1.69E-05
ENSG00000270344.2	RP11-734K2.4	0.73	9.31E-17
ENSG00000260257.2	RP5-1085F17.3	0.72	0.000169607
ENSG00000261226.1	RP11-830F9.7	0.63	7.69E-06
ENSG00000249859.7	PVT1	0.62	5.88E-46
ENSG00000271020.1	RP11-10C24.1	0.61	6.26E-05
ENSG00000247092.6	SNHG10	0.57	2.45E-12
ENSG00000232445.1	RP11-132A1.4	0.57	2.35E-06
ENSG00000271643.1	RP11-10C24.3	0.56	9.12E-08
ENSG00000262202.4	RP11-160E2.6	0.56	5.30E-09
ENSG00000259977.1	AL121578.2	0.53	7.72E-06
ENSG00000255774.1	AP000439.3	0.40	0.000181605
ENSG00000252690.3	SCARNA15	0.39	1.54E-08
ENSG00000255717.6	SNHG1	0.34	6.40E-61
ENSG00000236824.1	BCYRN1	0.32	4.03E-08
ENSG00000236830.6	CBR3-AS1	0.31	6.86E-12
ENSG00000226950.6	DANCR	0.30	7.63E-15
ENSG00000234912.9	SNHG20	0.25	3.78E-05
ENSG00000227051.5	C14orf132	0.23	3.24E-16

Table I. Continued.

Ensembl ID	LncRNA name	Fold change (log2)	FDR
ENSG00000235123.5	DSCAM-AS1	0.22	2.09E-29
ENSG00000163597.14	SNHG16	0.20	3.35E-46
ENSG00000259623.1	RP11-156E6.1	0.19	1.91E-06
ENSG00000260032.1	LINC00657	0.16	3.44E-37
ENSG00000224032.6	EPB41L4A-AS1	0.16	5.66E-06
ENSG00000242125.3	SNHG3	0.14	1.29E-05
ENSG00000244879.4	GABPB1-AS1	0.11	1.09E-05
ENSG00000234741.7	GAS5	0.06	6.73E-08
ENSG00000175061.17	LRRC75A-AS1	0.05	3.01E-05

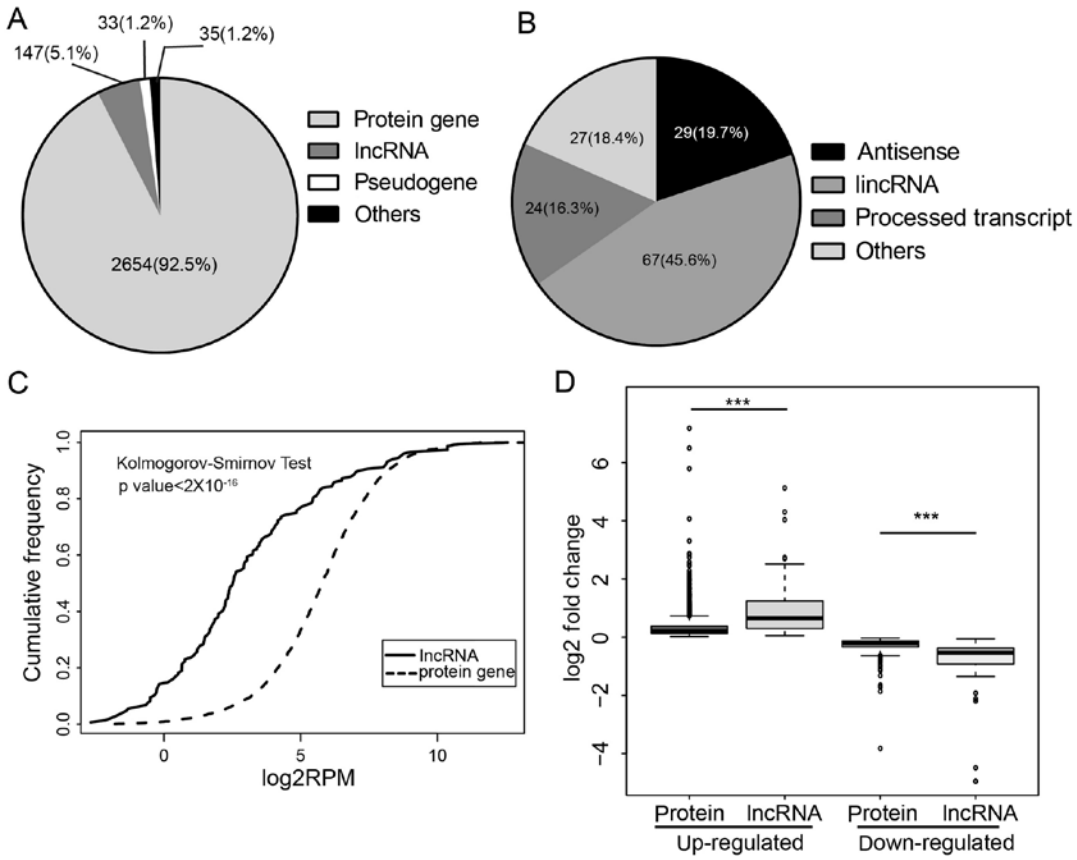

Figure 1. Summary of differential expressed lncRNAs upon E2 treatment. (A) Distribution of differential expressed transcriptions upon estrogen treatment. 'Others' include snoRNAs, snRNAs and miscRNAs. (B) Fraction of different classification of DE lncRNAs. (C) Cumulative frequency distribution showing expression levels of DE lncRNAs and protein-coding genes. RPM (reads per million) value are on log scale. (D) Boxplots showing fold change of DE lncRNAs compared to protein-coding genes. Significance calculated by Wilcoxon Rank-Sum test indicated by $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

referred to as putative ER-upregulated lncRNAs (Table I). To explore the potential roles of these lncRNAs, we conducted co-expression analysis between lncRNAs and reverse-phase protein array (RPPAs) proteomics data (30), which includes extensively validated antibodies to nearly 200 proteins and phosphoproteins of TCGA clinical samples. We found most of these lncRNAs co-expressed with some key regulators of the cell cycle (cyclin family) and apoptosis (Bcl-2 family) as well as key components of important signaling pathway such as IGF1R, MAPK (pT202/Y204) and JNK2. These data suggest that ER can transcriptionally regulate hundreds of lncRNAs that may participate in some important cellular processes.

Detection of ER-regulated lncRNAs. To verify the DE lncRNAs analyzed by RNA-seq, we selected six lncRNAs for further analysis. We first performed qPCR experiments after E2 treatment. Consistent with RNA-seq results. In MCF7, the most significantly changed lncRNA is *AP000439.3*, which increased 6 times. The expressions of this lncRNA in T47D was too low to be effectively detected. We also observed

Table II. Primers and siRNAs used in this study.

qPCR primers	Forward	Reverse
AP000439.3	CCCCAGGCTAGGAAGATGT	GAGCCAAGAGGTCCTCACAG
RP11-321G12.1	GGTTTGGTTCCCAATTGTTG	TTGGAAGACCCCATCTTCAC
RP11-150O12.3	ACCATTTCCAAACTGCCAAG	GCTCCATGCACACTCAAGAA
LINC01016	TACAGCATGGTTCCCAAATG	GGGCCATGGTCACTCATATT
SIAH2-AS1	CTCCTCAATCCCCACACAGT	TGCAGACGTGTATTCGGGTA
RP11-95G17.2	TTGCTGTAGTGCGGCTTAAA	TAACCCCTTGCAATCAGCTC
ChIP PCR primers	**Forward**	**Reverse**
ER binding site 1	AGGGAGAGTTCCCAGGAGTC	CAGCCCTGTCTGAGCAATTT
ER binding site 2	CTCCACCGAGCACTCCATAC	TGCCTCTTGTTTCCCCTAAA
siRNAs	**sense**	**antisene**
AP000439.3 siRNA 1#	GCUAGGAAGAUGUGCACCU	AGGUGCACAUCUUCCUAGC
AP000439.3 siRNA 2#	GCAAAGCUCACAGGAAAUA	UAUUUCCUGUGAGCUUUGC
ERα siRNA	CGAGUAUGAUCCUACCAGA	UCUGGUAGGAUCAUACUCG

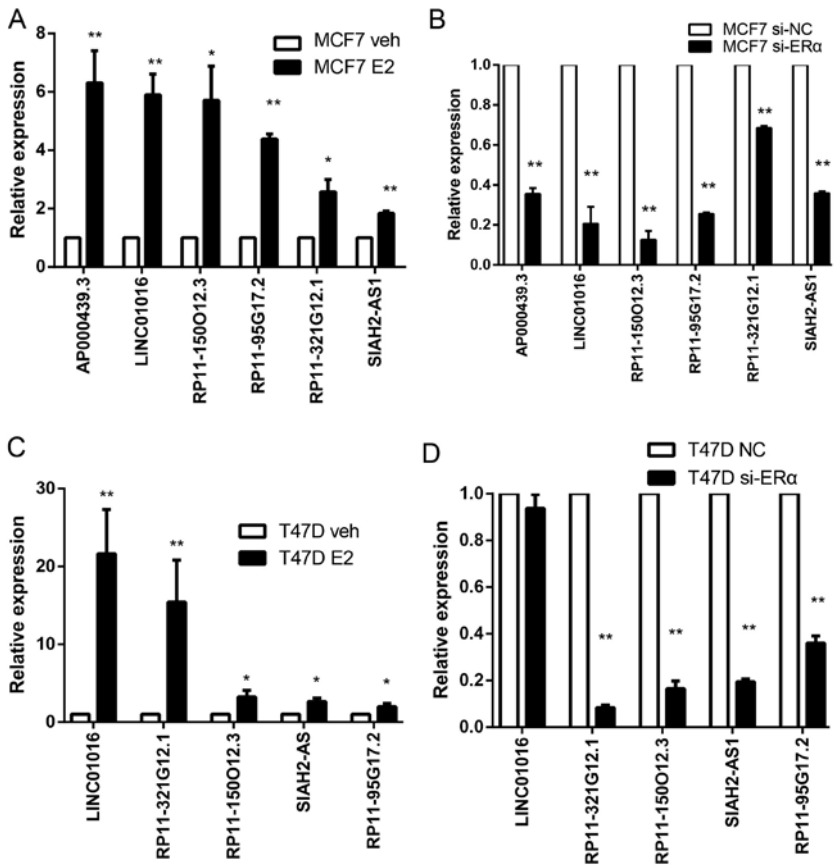

Figure 2. Expression change of ER target lncRNAs followed by either E2 treatment or knock down of ERα. (A) MCF7 cells were treated with 10 nM E2 or vehicle (ethanol) for 24 h and expression of lncRNAs were detected by qPCR. (B) MCF7 cells were transfected with either ERα or control siRNAs for 72 h and lncRNAs were detected by qPCR. (C) T47D cells were treated with 10 nM E2 or vehicle (ethanol) for 24 h and expression of lncRNAs was detected by qPCR. (D) T47D cells were transfected with ERα siRNAs for 72 h and lncRNAs were detected by qPCR. Significance calculated by Student's t-test is indicated by $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

significant increase of *AP000439.3* after E2 treatment in ZR-75-1 cells (data not shown). The other lncRNAs also increased remarkably after E2 treatment of both in MCF7 and T47D (Fig. 2A and C). To confirm that these DE lncRNAs

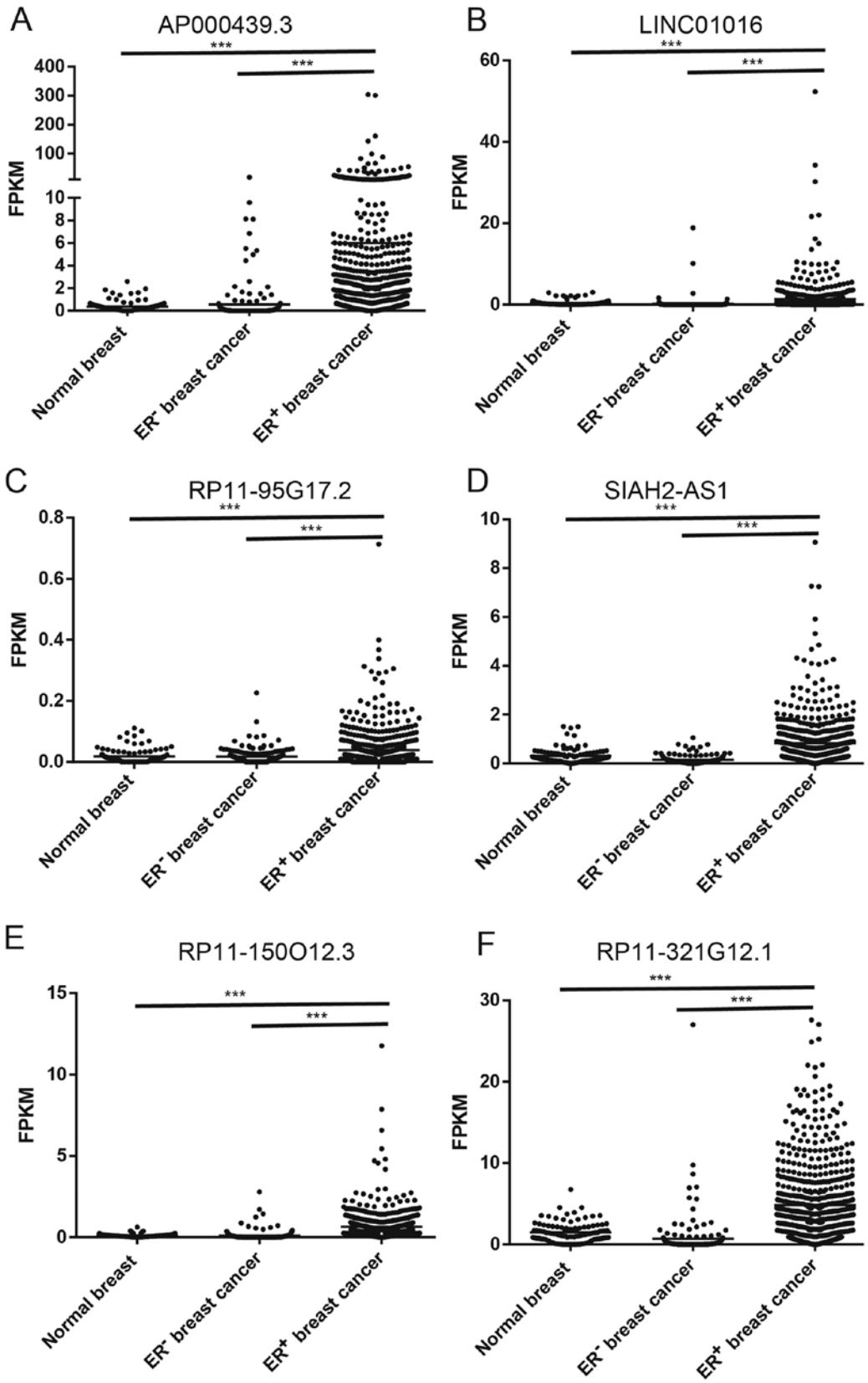

Figure 3. Expression of selected lncRNAs in clinical samples. The expression (FPKM) of these lncRNAs in breast cancer were shown by using TCGA data. Significance calculated by Wilcoxon rank-sum test indicated by $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

are regulated by ER, we silenced ERα in MCF7 and T47D by siRNA. In MCF7 cells, all of these lncRNAs dramatically decreased when silencing ERα, some of the lncRNAs such as *RP11-150O12.3, AP000439.3* and *RP11-95G17.2* decreased by >60% (Fig. 2B and D). Similar results were observed when ER was knocked down in T47D. Although *LINC01016* is a significantly upregulated lncRNA in T47D, silencing ER did not result in its reduction. *LINC01016* has been reported as

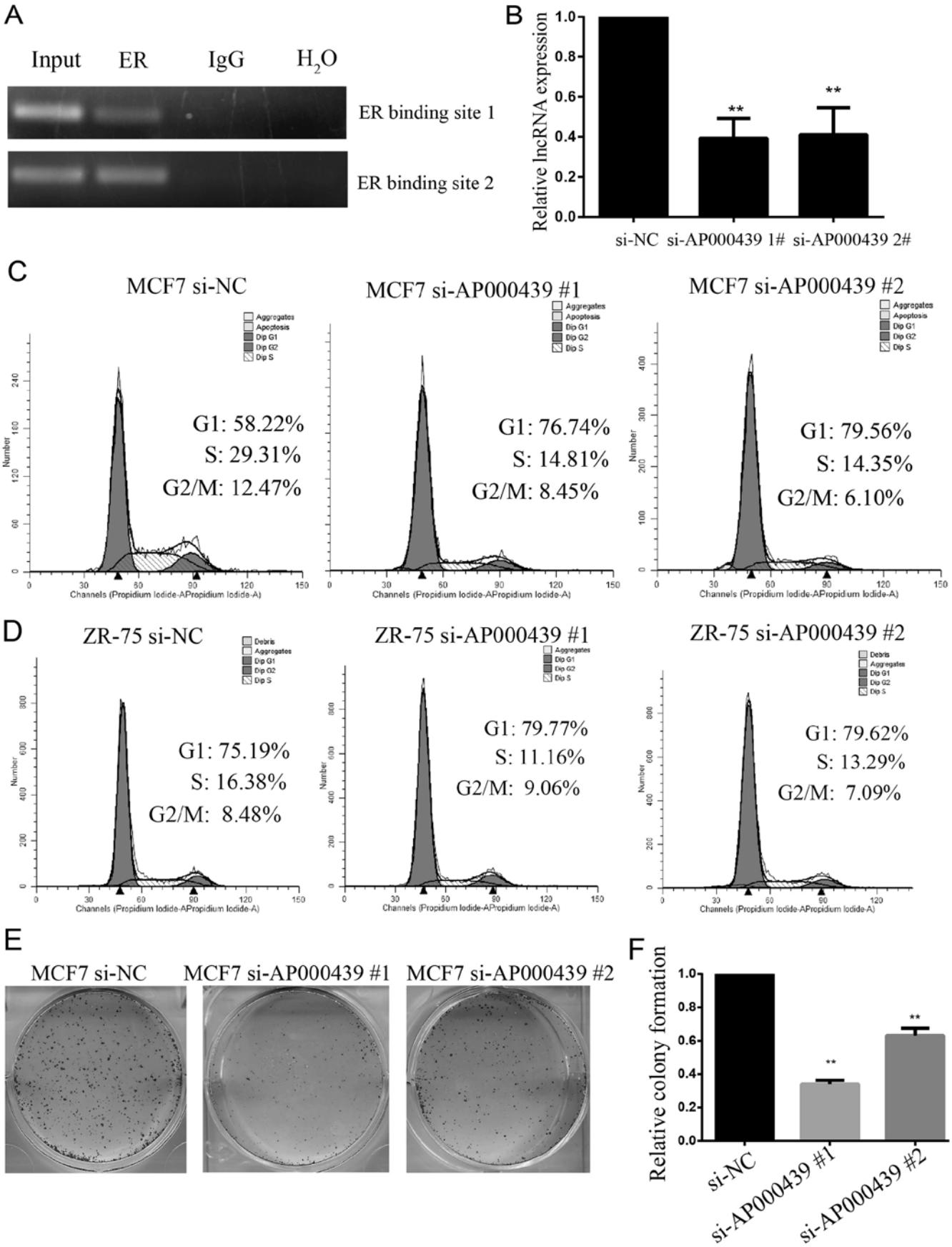

Figure 4. Silencing lncRNA *AP000439.3* results in inhibition of cell cycle progression. (A) ChIP assays show that ER can bind upstream of *AP000439.3*. MCF7 chromatin fragments were immunoprecipitated with antibodies for ER and negative control antibody (normal rabbit IgG). (B) qPCR experiments show the knock down efficiency of individual siRNAs of *AP000439.3*. (C) Flow cytometry assay determinate the effect of silencing AP000439.3 on cell cycle progression in MCF7 cells. (D) Flow cytometry assay determined the effect of silencing AP000439.3 on cell cycle progression in in ZR-75-1 cells. (E) Representative results of colony formation assays of MCF7 cells transfected with indicated siRNAs. (F) Graphic display of results of colony formation experiments, the numbers of colonies were normalized to control sample, Means ± SD of three independent experiments are shown.

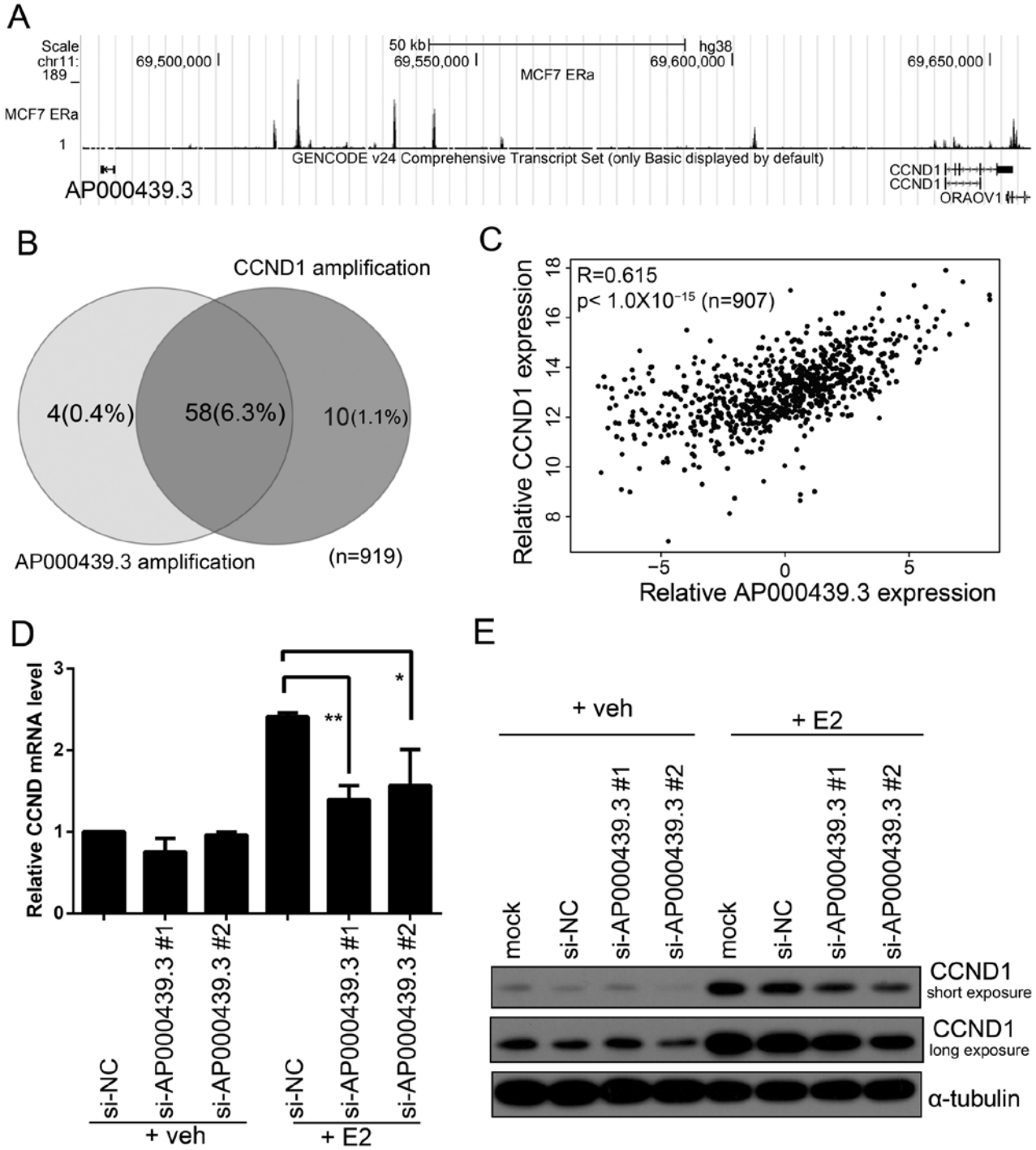

Figure 5. *AP000439.3* facilitates estrogen induced CCDN1 expression. (A) Genomic organization of *AP000439.3* and *CCDN1*. The direction of transcription are shown by the arrows, the ERα binding sites obtained by ChIP-seq are shown by the peaks. (B) Co-amplification of *AP000439.3* and *CCDN1*. Left circle showing *AP000439.3* amplified samples, right circle showing *CCDN1* amplified and overlapped region showing co-amplified samples (COSMIC CNV data, n=919). (C) Correlation between *CCDN1* and *AP000439.3* expression (TCGA data). Pearson's product moment correlation coefficient was used to determinate association between *CCND1* and *AP000439.3*. (D) MCF7 were cultured in estrogen deprived medium and transfected with either *AP000439.3* or control siRNAs for 48 h, then treated with 10 nM E2 for 8 h. *CCND1* mRNA level were detected by qPCR. (E) MCF7 were treated the same as (D), protein was analyzed by western blotting.

an ER-target lncRNA in a previous study (13,14). Failure to observe a decrease of *LINC01016* when silencing ERα may be due to its low expression level in T47D, hence hard to down-regulate further, but easy to upregulate.

ER-regulated lncRNA AP000439.3 promotes cell cycle progression. To further characterize these ER-regulated lncRNAs, we analyzed the expression of lncRNAs in breast tissues using The Cancer Genome Atlas (TCGA) data (31). Most of these selected lncRNAs were dramatically overexpressed in ER^+ breast cancer compared to ER^- breast cancer and normal breast tissues ($p<0.001$, Fig. 3).

To explore the roles of ER target lncRNAs in breast cancer, we selected one of the most significantly upregulated lncRNA

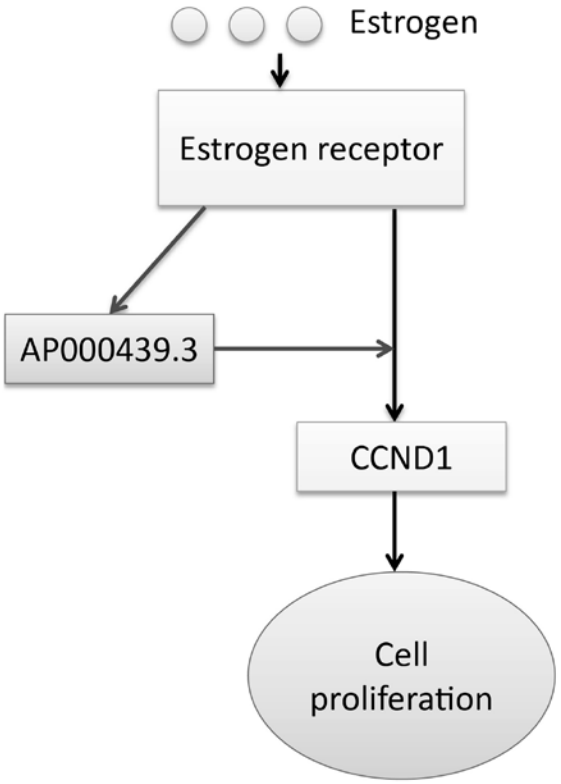

Figure 6. Model of regulation and roles of lncRNA *AP000439.3*. *AP000439.3* is transcriptionally regulated by ER, *AP000439.3* can improve the expression of *CCND1* induced by estrogen and thus promote cell cycle progression and cell proliferation.

(AP000439.3) for further analysis. Using ChIPBase, an integrated database for decoding transcriptional regulators of genes and lncRNAs (32,33), we found ERα is the top ranked regulator. To verify that AP000439.3 is regulated by ER, ChIP PCR assay was performed on potential ER binding sites identified by ChIP-seq data and ChIPBase. As shown in Fig. 4A, ER can directly bind upstream of *AP000439.3*.

Since ER can regulate dozens of genes that play crucial roles in breast cancer, we speculate these lncRNAs may also have an important impact on breast cancer cell function. AP000439.3 was silenced using two individual siRNAs by >50% (Fig. 4B). The most dominant role of ER in breast cancer is promotion of cell proliferation and cell cycle progression (1). Hence the cell cycle progression was detected using flow cytometry assay. Silencing *AP000439.3* resulted in a dramatic inhibition of the G1-S transition in MCF7 cells (Fig. 4C) and in ZR-75-1 cells (Fig. 4D). Likewise, silencing *AP000439.3* significantly suppressed clonogenic proliferation (Fig. 4E and F). Taken together, lncRNA *AP000439.3* is stimulated by activated ER, it is overexpressed in ER^+ breast cancer and promotes cell cycle progression and proliferation.

AP000439.3 facilitates estrogen induced CCDN1 expression. To understand how AP000439.3 regulates the cell cycle, we first investigated its location and gene structure. *AP000439.3* is a long intergenic non-coding RNA (lincRNA) located at chromosome 11q13. *AP000439.3* is 160 kb upstream of the *CCDN1* transcriptional start site (TSS) (Fig. 5A), *AP000439.3* and *CCDN1* are transcribed divergently (head to head). *CCND1* encodes the cyclin D1 protein that serves as a regulator of cyclin-dependent kinase as a crucial regulator of the cell cycle. Amplification of *CCND1* has been reported in many kinds of tumors including breast cancer, its amplification is associated with a poor prognosis (34). Analysis of COSMIC CNV data (35) revealed that CCND1 amplification occurred in 68/919 (7.4%) breast cancer samples, 58 of 68 (85%) of these also have an amplification of *AP000439.3* (Fig. 5B). Expression analysis of *CCND1* and *AP000439.3* showed a high correlation between them ($R=0.61$, $p<10^{-15}$, Fig. 5C). *CCND1* is overexpressed in ~50% breast cancer, while amplification of the *CCND1* gene is present only in a minority of *CCND1*-overexpressed breast cancers (36), directly regulated by ER is another important reason for overexpression of *CCND1* (37).

LncRNAs are likely to regulate the expression of an adjacent gene (38-40). Hence we speculated that *AP000439.3* may influence the expression of *CCND1*. We silenced *AP000439.3* in MCF7 and then treated these cells with either E2 or vehicle. Both qPCR and western blotting showed that knockdown of *AP000439.3* could impair *CCND1* expression induced by E2 (Fig. 5D). Taken together, these data suggest ER-regulated lncRNA *AP000439.3* can facilitate ER regulation of *CCND1* and thus promote cell cycle progression (Fig. 6).

Discussion

In this study, we described a genome-wide identification and characterization of ER-regulated lncRNAs in breast cancer cells. We found many of these lncRNAs were overexpressed in ER^+ breast cancer and co-expressed with some key regulators. Moreover, we found one of the most prominent lncRNA, *AP000439.3*, can promote cell cycle progression through enhancing *CCDN1* expression induced by estrogen.

The mechanisms of how *AP000439.3* facilitate *CCND1* expression remains to be further investigated. Considering *AP000439.3* can influence transcription of *CCND1*, it may involve changes in chromatin organization. This mechanism have been reported by lncRNA CCAT1-L regulation on its adjacent gene C-MYC in colorectal cancer (41).

The roles of ER is mediated primarily by its downstream effectors, the ER-regulated protein-coding genes and their roles have been well studied. However, some important issues such as the mechanisms by which ER-mediated drug resistance remains largely unknown (42). Study of ER-regulated lncRNAs may provide a unique prospective to answer these questions.

Our findings may provide another layer of estrogenic control of gene expression: ER can promote expression of lncRNAs that are adjacent to protein-coding genes; these lncRNAs can serve as positive regulators that further facilitate transcriptional regulation by ER. It remains to be further investigated how many lncRNAs function in this way and whether these lncRNAs specifically regulate their neighboring adjacent genes or have a more extensive impact on ER regulation. Since these ER-regulated lncRNAs showed highly cell-type specificity, some oncogenic lncRNAs can be potential biomarkers and therapeutic targets.

Acknowledgements

We would like to thank Ms. Qing Wei and Ms. Fang Su for flow cytometry analysis. We thank The Cancer Genome Atlas (TCGA) project and their contributors for providing this valuable public data set. This study was supported by grants from the Natural Science Foundation of China (81572484, 81420108026 and 81621004 to D.Y.); Guangdong Science and Technology Department (2014A050503026 to D.Y., 2015B050501004 and S2012030006287); Guangzhou Bureau of Science and Information Technology (201704030036 to D.Y. This study was also supported by the RNA Biology Center at the Cancer Science Institute of Singapore, NUS, as part of funding under the Singapore Ministry of Education's Tier 3 grants, grant no. MOE2014-T3-1-006.

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