Super-Enhancer LncRNA LINC00162 Promotes Progression of Bladder Cancer

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HIGHLIGHTS
Expression of LINC00162 is increased in bladder cancer
LINC00162 promotes bladder cancer progress in vitro and in vivo
LINC00162 regulates neighboring PTTG1IP expression to promote bladder cancer
LINC00162 inhibits PTTG1IP expression by interacting THRAP3
Super-Enhancer LncRNA LINC00162 Promotes Progression of Bladder Cancer

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SUMMARY
Due to the lack of effective early diagnostic measures and treatment methods, bladder cancer has become a malignant tumor that seriously threatens people’s lives and health. Here, we reported that LINC00162, a super-enhancer long non-coding RNA, was highly expressed in bladder cancer cells and tissues. And LINC00162 was negatively correlated with neighboring PTTG1IP expression. Knocking down LINC00162 expression can inhibit the proliferative activity of bladder cancer cells and the growth of transplanted tumors in vivo, while knocking down the expression of PTTG1IP could restore the proliferative activity of bladder cancer cells. In addition, both LINC00162 and PTTG1IP were found to be able to bind to THRAP3, a transcription-related protein. And THRAP3 can regulate PTTG1IP expression. Finally, we demonstrated a mechanism that LINC00162 could regulate PTTG1IP expression through binding THRAP3. This study provided a potential target molecule for clinical treatment of bladder cancer.

INTRODUCTION
Bladder cancer is a common malignant tumor of the urinary system. In 2019, there were an estimated 80,470 cases of bladder cancer in the United States, and there will be 17,670 deaths (Siegel et al., 2019). Bladder cancer is one of the ten most common malignant tumors. It has the highest incidence in the urinary system. Increasing morbidity and mortality each year has seriously threatened people’s lives and health. However, with the rapid development of molecular diagnostic technology, a large number of tumor markers have been discovered, and the development of molecular targeted drugs has become an effective measure for tumor treatment. Therefore, the exploration of novel target molecules for bladder cancer has important clinical significance for the treatment of bladder cancer.

In recent years, noncoding RNA has gradually become a hot topic of research in medical research, and many studies have proved that noncoding RNA could participate in regulating the pathological processes of various cancers (Huang et al., 2016; Liang et al., 2018; Yuan et al., 2014; Zhang et al., 2019). There are 3 billion base pairs in the genome but only about 30% of transcripts derived from the genome can encode proteins, the remaining about 70% are noncoding RNA (Atianand and Fitzgerald, 2014; Parasarman et al., 2016). Although the number of noncoding RNA is much greater than that of coding RNA. However, the research on the biological function of noncoding RNA is still limited. Long noncoding RNA (IncRNA) is a type of long noncoding RNA longer than 200nt (Zhang et al., 2017). Although IncRNA itself does not have the function of encoding proteins, it could participate in regulating various cell physiological activities such as cell differentiation, proliferation, apoptosis, and metastasis (Xie et al., 2017). At present, studies have shown that IncRNA is abnormally expressed in many tumors and has become a potential target molecule for many cancers (Kong et al., 2015; Tan et al., 2018; Xiao et al., 2018; Xie et al., 2018). Moreover, IncRNA could also regulate the occurrence and development of malignant tumors through various pathways. For example, IncRNA NORAD competitively adsorbs hsa-miR-125a-3p through the sponge function and then regulates the expression of RhoA, thereby promoting the development of pancreatic cancer (Li et al., 2017). IncRNA TROJAN accelerates the degradation of ZMYND8 through the ubiquitination pathway and subsequently promotes the progression of breast cancer (Lin et al., 2019). IncRNA TUG1 could bind to the enhancer of EZH2, regulating the expression of LIMK2b, and then promote the cell growth and
We found that the expression of LINC00162 was most significantly upregulated in the super-enhancer IncRNA microarray expression profile. Compared with normal bladder epithelial cells and tissues, LINC00162 also had significant differences in bladder cancer cells and tissues. This study mainly explored the function of LINC00162 in the development of bladder cancer. We found that knocking down the expression of LINC00162 could effectively inhibit the proliferative viability of bladder cancer cells, the ability to form clones in soft agar, and the rate of tumor growth in nude mice. Therefore, LINC00162 plays a tumor-promoting function in bladder cancer. By studying the mechanism of LINC00162 regulating the proliferation of bladder cancer cells, we found that LINC00162 could inhibit the neighboring PTTG1P expression through binding to THRAP3, a transcriptional regulatory protein, and finally promote the proliferation of bladder cancer cells. This discovery not only allowed us to understand the function and mechanism of LINC00162 in bladder cancer but also provided a potential target molecule for the treatment of bladder cancer.

RESULTS
Detection of LINC00162 Expression in Bladder Cancer Cells and Tissues
We used the immortalized normal bladder epithelial cell line SV-HUC-1 and bladder cancer cell UM-UC-3 as cell samples for super-enhancer IncRNA microarray analysis. We used fold changes in microarray analysis results to show differences in IncRNA expression (Figure 1A). Specific primers were designed to verify differentially expressed SE IncRNA between SV-HUC-1 and UM-UC-3 cells. And we verified five upregulated SE IncRNAs in UM-UC-3 cells by quantitative polymerase chain reaction (q-PCR) test, of which LINC00162 has the highest fold change (Figure 1B). So, LINC00162 was selected out to further explore its biological function and mechanism in vivo and in vitro. LINC00162 is composed of two exons of P38 inhibited cutaneous squamous cell carcinoma associated lincRNA (PICSAR) (Figure 1C). Then, we detected the expression of LINC00162 in SV-HUC-1, UM-UC-3, and T24 cells. Compared with SV-HUC-1 cells, the expression of LINC00162 in UM-UC-3 and T24 was significantly upregulated (Figure 1D). We also tested the expression of LINC00162 in 32 pairs of bladder cancer tissues and adjacent tissues. Compared with adjacent tissues, LINC00162 was upregulated in bladder cancer tissues (Figures 1E and 1F). Subsequently, we used receiver operating characteristic curve (ROC) curve analysis and evaluation. The results showed that the area under the curve was 0.709 (95% confidence intervals (CI): 0.579–0.839), indicating that LINC00162 has a potential diagnostic value (Figure 1G).

LINC00162 Promotes Proliferation of Bladder Cancer Cells
In order to initially predict the potential functions of LINC00162, we analyzed the RNAs regulated by LINC00162 and its pathways with bioinformatics methods. In brief, we first predicted the miRNA that LINC00162 might bind to through the RegRNA 2.0 (http://regrna2.mbc.nctu.edu.tw/) and then predicted the mRNA that these miRNAs may bind to through the TargetScan (http://www.targetscan.org/vert_71/). Then, we conducted pathway enrichment analysis on these mRNAs and found that LINC00162 was involved in a variety of tumor-related pathways and was also enriched in pathways such as protein binding and biological regulation (Figures 2A and S1A–S1C). To further investigate whether LINC00162 is involved in the development of bladder cancer, we designed the siRNA of LINC00162 and constructed its overexpression plasmid and performed efficiency verification in UM-UC-3 and T24 cells (Figure S1D). Subsequently, to study the effect of LINC00162 on cell physiology, we silenced or overexpressed LINC00162 and found that various physiological behaviors of cells changed significantly, such as cell proliferation (Figures 2B and 2C), cell apoptosis (Figure 2D), and cell cycle (Figure 2E). Compared with the control group, UM-UC-3 and T24 cells transfected with the siRNA group (LINC00162 siRNA1 or LINC00162 siRNA2) significantly reduced cell viability, inhibited cell proliferation, promoted apoptosis, and increased cells in the G0/G1 block. Compared with the vector group, UM-UC-3 and T24 cells transfected with the LINC00162 overexpression plasmid significantly increased cell viability, promoted cell proliferation, inhibited apoptosis, and reduced G0/G1 phase block (Figures 2B–2E). In order to investigate the effect of LINC00162 in vivo, we constructed a stable knockdown cell line of LINC00162 shRNA using UM-UC-3 cells and tested the silencing efficiency of stable cells by q-PCR (Figures S2A–S2C). The number of monoclonal cells in the LINC00162 shRNA group was significantly reduced compared with the control group in the soft agar clone formation experiment (Figure 2F). Next, we studied the effect of LINC00162 on tumor-bearing in
nude mice. LINC00162 shRNA stable cell lines and vector stable cell lines were subcutaneously injected into nude mice, and the tumors were removed after 21 days for observation and weighing (Figure 2G). We found that tumors formed by LINC00162 shRNA stable cell lines were significantly decreased compared to the control group.

**LINC00162 Affects Neighboring Gene Expression**

To illustrate the distribution of LINC00162 at the subcellular level, we performed fluorescence in situ hybridization experiments (FISH) in UM-UC-3 and T24 cells. As shown in Figure 3A, LINC00162 is mainly expressed in the nucleus. By comparing the chromosomal position of LINC00162, it was found that the...
sequence encoding LIN00162 overlaps with the segment encoding the super-enhancer. To investigate whether LIN00162 can regulate neighboring gene expression, we selected neighboring genes in the range of 250kb upstream and downstream of LIN00162, respectively. UBE2G2, SUMO3, PTTG1IP, ITGB2, FAM207, ADARB1, POFUT2 (Figure 3B). Subsequently, we designed an RNA-specific probe for LIN00162, and according to the requirements of the chromatin isolation by RNA purification (CHIRP) experiment (Figure 3C), it ultrasonically shattered the whole genome DNA in UM-UC-3 and T24 cells. So, the content of the fragment was more than 70% at 100bp-500bp (Figure 3D). Next, the DNA fragment bound to LIN00162 was pulled down through the CHIRP experiment. q-PCR experiments showed that compared with other neighboring genes, PTTG1IP was pulled down by LIN00162 probes (Figures 3E and 3F). To determine whether there was an expression correlation between LIN00162 and neighboring genes, we used LIN00162 siRNA1 and siRNA2 to silence the expression of LIN00162 RNA. The transient LIN00162 OE plasmid was used to overexpress LIN00162 expression. As shown in Figures 3G and 3H, the correlation between PTTG1IP and LIN00162 expression was highest in UM-UC-3 and T24 cells, and it showed a significant negative correlation, suggesting that LIN00162 is an upstream gene of PTTG1IP and regulates the expression of PTTG1IP. In order to further determine the regulatory role of LIN00162 on PTTG1IP in vivo, we fixed, embedded, and sliced the tumors formed in nude mice subcutaneous tumor experiments and performed immunohistochemical experiments to detect the expression of PTTG1IP. As shown in Figure 3I, the expression of PTTG1IP protein was increased in the LIN00162 knockdown tumor bearing results in nude mice subcutaneous tumor-bearing results in nude mice at 27 days, *p < 0.05.

LIN00162 Promotes Proliferation of Bladder Cancer Cells by Regulating PTTG1IP

To investigate whether PTTG1IP is involved in the LIN00162 mechanism that promotes the proliferation of bladder cancer cells, we first designed and synthesized siRNA for PTTG1IP mRNA and verified the knockdown efficiency of siRNA on PTTG1IP protein level (Figure 4A). Next, we determined the effects of LIN00162 and PTTG1IP on cell proliferation (Figure 4B), apoptosis (Figure 4C), and cycle (Figure 4D) by flow cytometry and measuring total adenosine triphosphate (ATP) content. Silencing the expression of LIN00162 alone could reduce the proliferation of bladder cancer cells, increase the apoptosis of bladder cancer cells, and block the G0/G1 phase. But silencing PTTG1IP expression alone could promote the proliferation of bladder cancer cells, reduce the apoptosis of bladder cancer cells, and promote G0/G1 phase. Compared with the control group, silencing the expression of LIN00162 combined with silencing the expression of PTTG1IP showed no statistically significant changes in bladder cancer cell proliferation, apoptosis, and cycle. These results showed that PTTG1IP could inhibit the proliferation of bladder cancer, promote cell apoptosis, and block G0/G1 phase and that LIN00162 could inhibit the regulation of PTTG1IP.

LIN00162 Regulates PTTG1IP by Binding to THRAP3

In order to investigate how LIN00162 regulates the expression of PTTG1IP, we used RNA pull-down experiments and mass spectrometry to investigate whether LIN00162 regulates PTTG1IP by recruiting transcription-related proteins. To this end, we designed an RNA pull-down specific probe for LIN00162 and performed an RNA pull-down experiment in the UM-UC-3 cell line (Figure 5A). Silver staining results showed that there were a variety of proteins that could bind to the LIN00162 molecule, the molecular weight distribution of the protein was relatively wide, and the protein enrichment was highest at 40kd-55kd and 100kd (Figure 5B). After protein purification, many related proteins were obtained by mass spectrometry analysis, and most of the proteins had binding functions (Figures 5C and S3A–S3C). We screened
multiple transcription-related proteins and found that THRAP3 had the highest binding index with PTTG1IP DNA nucleotide (Figure 5D). We analyzed the molecular function of THRAP3 through gene ontology (GO) annotations and found that THRAP3 could participate in multiple transcriptional regulatory pathways (Figure 5E). To ensure the protein identity of THRAP3, we derived the protein map of THRAP3 using mass spectrometry (Figure S3D) and tested the content of THRAP3 protein in the input group, the RNA pull-down (RPD) group, and the negative control (NC) group by Western blot experiments. As shown in Figure 5F,
Figure 4. LINC00162 Promotes the Proliferation of Bladder Cancer Cells by Regulating PTTG1IP
(A) Western blot verified the efficiency of PTTG1IP silencing.
(B) Effects of LINC00162 and PTTG1IP on the viability of UM-UC-3 and T24 cells detected by the ATP method, *p < 0.05.
(C) Effect of LINC00162 and PTTG1IP on apoptosis of UM-UC-3 and T24 cells. Annexin Fluorescein isothiocyanate isomer (FITC) and propidium iodide (PI) are used to indicate apoptosis, *p < 0.05.
(D) The effects of LINC00162 and PTTG1IP on the cell cycle of UM-UC-3 and T24. Experimental cells were stained with propidium iodide (PI). Count the number of cells at different cell cycle stages, *p < 0.05.
Figure 5. LINC00162 Regulates PTTG1IP by Binding to THRAP3

(A) Schematic of RNA pull-down experiment.
(B) Silver-stained analysis of RNA pull-down experimental samples (RNA-binding proteins).
(C) The RNA-binding proteins detected by mass spectrometry were analyzed by Gene Ontology.
(D) Prediction of THRAP3 and PTTG1IP mRNA binding. Interaction tendencies and discrimination in the diagrams represent the possibility and ability of interaction, respectively.
(E) Gene Ontology annotations of THRAP3.
(F) Western blot was used to detect the protein content of THRAP3 in the input group, RPD group, and NC group in the pull-down experimental samples.
(G) The effect of changes in THRAP3 protein on the expression of PTTG1IP protein. THRAP3 siRNA was transfected into UM-UC-3 cells and T24 cells, respectively. Cell protein was collected 48 hr later, and the protein levels of THRAP3 and PTTG1IP were detected by Western blot experiments.
(H) Schematic of RIP experiment.
(I and J) Agarose gel electrophoresis was used to detect the content of LINC00162 or PTTG1IP mRNA in the input group, IP group, and IgG group in RIP experiments.
the THRAP3 protein could indeed be pulled down by the specific molecular probe of LINCO0162, and it proved that THRAP3 could bind to LINCO0162 again. To investigate the correlation between THRAP3 and PTTG1IP, we performed THRAP3 expression silencing in two bladder cancer cell lines (UM-UC-3 and T24) and collected the proteins from the two cell lines for Western blot detection. As shown in Figure 5G, after the THRAP3 protein expression was downregulated, the PTTG1IP protein expression also decreased accordingly, suggesting that THRAP3 could positively regulate PTTG1IP expression. In order to further prove the binding relationship of THRAP3 with LINCO0162 and PTTG1IP, we used the THRAP3 antibody to pull the bound molecule through RNA Immunoprecipitation (RIP) experiments (Figure 5H) and then performed PCR to detect candidate molecules. The contents of LINCO0162 and PTTG1IP DNA in the input group, Immunoprecipitation (IP) group, and IgG group were showed by agarose gel electrophoresis (Figures 5I and 5J). The results indicated that the contents of LINCO0162 and PTTG1IP DNA in the IP group were significantly higher than those in the IgG group, showing that THRAP3 has a binding effect with LINCO0162 and PTTG1IP.

DISCUSSION
Bladder cancer is a malignant tumor with the highest incidence in the urogenital system (Jeronimo and Henrique, 2014; Robertson et al., 2017). Because early symptoms are not obvious, most patients develop bladder cancer that reaches the middle or late stage when diagnosed, and this has greatly hindered clinical treatment (Antoni et al., 2017; Maia et al., 2018; Morales et al., 2016). Some progress has been made in bladder cancer research, and a large variety of tumor molecular targets have been found at the molecular level, such as genes and proteins (PS3, Ki-67), providing important references for early diagnosis and treatment of bladder cancer (Bazrafshani et al., 2016; Noel et al., 2015; Onal et al., 2015; Puntoni et al., 2016). However, these molecular tumor targets that are used currently still fail to meet the ideal requirements in terms of sensitivity or specificity. Therefore, more effective tumor molecular targets are urgently needed for clinical diagnosis and treatment support, and research on new target molecules in bladder cancer is extremely important. LncRNAs are an important constituent member of the endogenous RNAs regulatory network (Zhang et al., 2018a, 2018b). In recent years, increasing evidence has suggested that lncRNAs can participate in the regulation of various levels of chromosome modification, RNA transcription, and protein translation in physiological cell processes (Hon et al., 2017; Park et al., 2018; Podbevsek et al., 2018). In addition, lncRNAs can also have important effects on various stages of cell physiology (Misawa et al., 2016; Rosman et al., 2019; Xu et al., 2019). At the present time, a large amount of literature has shown that lncRNAs can regulate the development of bladder cancer in various ways. For example, lncRNA MAGI2-AS3 adsorbs miRNA through the sponge function and then regulates the protein expression of CCDC19, thereby inhibiting the development of bladder cancer (Wang et al., 2018). LncRNA DUXAP8 promotes the proliferation of bladder cancer cells by inhibiting the expression of PTEN protein (Lin et al., 2018). LncRNA ELF3-AS1 interacts with KLF8 protein molecules to form a positive feedback loop, thereby promoting the progress of bladder cancer (Guo et al., 2019).

LINC00162 is a newly discovered potential molecular target for bladder cancer, located at 21q22.3. It has been reported in skin cancer and rheumatoid arthritis (Bi et al., 2019; Pipponen et al., 2018), but its biological role and clinical significance in bladder cancer have not yet been elucidated. In this study, we observed that LINC00162 expression in bladder cancer cell lines and bladder cancer tissues was significantly upregulated. Further experiments proved that knocking down the expression of LINC00162 significantly inhibited the proliferative activity of bladder cancer cells and the ability to form monoclonal colonies in soft agar, as well as the tumor growth in nude mice, and at the same time reduced the apoptosis rate. Further, upregulating the expression of LINC00162 would produce the opposite cell phenotype change. It is suggested that LINC00162 may play a role as an oncogene in the development of bladder cancer. To clarify the molecular mechanism of LINC00162 regulating bladder cancer cell proliferation, we used CHIRP experiments to prove that LINC00162 could bind to its neighboring gene, PTTG1IP. Previously, PTTG1IP was reported to participate in the regulation of cancer development such as non-small cell lung cancer, breast cancer, glioma, and thyroid cancer through various methods such as high methylation, increasing ubiquitination, and regulating the cell cycle (Read et al., 2014; Repo et al., 2017; Tan et al., 2019; Wang et al., 2014). However, the biological function of PTTG1IP in bladder cancer has not been reported. This study is the first to explain the function of PTTG1IP in bladder cancer. RNA-FISH experiments were used to detect the subcellular localization of LINC00162. The results show that LINC00162 is mainly distributed in the nucleus, suggesting that LINC00162 may play a role in the transcription process. Through further experiments, we found that LINC00162 could regulate the expression of PTTG1IP, and these two
molecules are also closely related in cell function. In order to explore the molecular mechanism of LINC00162 regulating PTTG1IP, we used RNA pull-down experiments to pull down the proteins that bind to LINC00162. And by mass spectrometry analysis, we screened one of the most abundant transcription-related proteins, THRAP3. THRAP3 is involved in the transcriptional regulation of multiple pathways as a transcriptional regulation factor. For example, it participates in the formation of the SOX9 transcription complex and negatively regulates the transcriptional activity of SOX9 (Sono et al., 2018). THRAP3 also works in concert with HELZ2 to enhance PPARG-mediated transcriptional activation (Lande-Diner et al., 2013). Through RIP experiments, we used THRAP3 antibodies to enrich nucleic acid molecules that bind to THRAP3 protein and found that both LINC00162 and PTTG1IP could bind to THRAP3 protein. Further Western blot experiments proved that THRAP3 had a positive regulation effect on the protein expression of PTTG1IP. Based on the above results, it is suggested that LINC00162 may block the pathway that THRAP3 promotes the transcription of PTTG1IP, thereby inhibiting the expression of PTTG1IP and then promoting the proliferation of bladder cancer cells. However, the specific molecular mechanism of the interaction between LINC00162, THRAP3, and PTTG1IP needs further study.

Our research shows that LINC00162 is an oncogene in the development of bladder cancer, and it inhibits PTTG1IP expression by interacting with THRAP3, thereby promoting the proliferation of bladder cancer cells (Figure 6). This study allowed us to understand the molecular function of LINC00162 more comprehensively. Moreover, we achieved a preliminary exploration of the regulatory mechanism of THRAP3 and PTTG1IP in bladder cancer. Our work provides basic support for the discovery of potential target molecules of bladder cancer.

Limitations of the Study

The specific experimental mechanism on how LINC00162 and THRAP3 complex inhibit PTTG1IP expression has not been fully elucidated.

Resource Availability

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Aruo Nan (nanaruo@163.com).
Materials Availability
This study did not generate new unique reagents.

Data and Code Availability
The accession number for the Super-enhancer IncRNA microarray dataset reported in this paper is GEO: GSE159682. Other data are included in the published article and the supplemental information files and any additional information will be available from the lead contact upon request.

METHODS
All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101857.

ACKNOWLEDGMENTS
We thank Dr. Dapang Rao, the Second Affiliated Hospital & Yuying Children’s Hospital of Wenzhou Medical University, Wenzhou, Zhejiang 325027, China, for critical technical support. This work was supported by the National Natural Science Foundation of China (NSFC81903356, NSFC81673135).

AUTHORS CONTRIBUTION
A.R.N. and H.S.H. conceived of the study. X.W., R.R.Z., and S.L.W. performed the experiments. L.P.S., M.X.K., Y.O.Y., M.Q.L., Z.J.Z., Y.P.Y., and W.M.L. participated in statistical analysis of data, result arrangement and drawing, and manuscript writing. J.L.Y., J.Y., and D.N.L. collected clinical samples. A.R.N. and Y.F.Z. revised the paper. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.

Received: July 20, 2020
Revised: October 21, 2020
Accepted: November 19, 2020
Published: December 18, 2020

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Supplemental Information

Super-Enhancer LncRNA LINC00162
Promotes Progression of Bladder Cancer

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### Table S1. Information of the qPCR primer sequences, Related to Figure 1.

| Primer (for qPCR) | Forward primer (5’ to 3’) | Reverse primer (5’ to 3’) |
|------------------|---------------------------|--------------------------|
| LINC00162        | ACTGCCTGGGACTTTCAAGAGG    | CCTCTTGGGTAGGGGTGCC      |
| RP11-152P17.2-007| AGGCTTCTCGCATAGCTTCT      | ACTCCATACTCTCCCTGAGTT    |
| RP11-548L20.1-001| ACAGTGACAAGGGACCGAGATT   | TGGAGTATCGCAAGAACAGTGG   |
| RP11-527H14.3-001| GGTAGCATCGCAGTCACT        | TCTCTTCCTCGGTCTTTCCT     |
| RP11-152P17.2-001| AGGAAGTGTGGACAGAAGATGAG  | AATGACTCCCTCAAGGGTACG    |
| PTTG1IP          | CCAGTTACAGCGCTTTGCC       | CGGCTCAAGGTCACCCCAAC     |
| ADARB1           | TAGAGTCTGGTGGAGGGAGC      | GGAGATCCCGTCGGTCATG      |
| UBE2G2           | GTACTGAACGCAGCCTCCGT      | AAGATGCCAGTCTCTCCCCT     |
| SUMO3            | TCAGATCAGGTCGGACGGG       | ACAATCCGAAAGTCGCCCTG     |
| ITGB2            | GTGCAGTTCAGGAGGCCCTTC     | CCCGGGAAACTCTCTGTCTATG   |
| POFUT2           | CGGAGGGGTTCCGCTTATTG      | AACCTTTGGAAGCCACCCGA     |
| FAM207           | CAGCAGGGGAGCAAAGAGG       | TCCTGAAACCGGGGTCTTCTTCC |
| GAPDH            | ATCAATGGGAATCCCATACCA     | GACTCCACGACGTACTCAGCG    |

### Table S2. RNA oligonucleotide sequences of siRNAs, Related to Figure 4.

| RNA oligos       | Sequences                                      |
|------------------|-----------------------------------------------|
| siRNA-NC         | Sense: 5’- UUCUCGAACGUGUCACGUTT -3’           |
|                  | Anti-sense: 5’- ACGUGACACGUUCGGAGAATT -3’     |
| LINC00162 siRNA1 | Sense: 5’- GCCUCUUCCUCAGAUCUTT -3’            |
|                  | Anti-sense: 5’- AGAUGUCUGAGGAAGAGCCT -3’      |
| LINC00162 siRNA2 | Sense: 5’- CGCCUCAGUUUCAGAUUGATT -3’          |
|                  | Anti-sense: 5’- UCAAUCUGAAAACUGCAGCGT -3’      |
| PTTG1IP siRNA1   | Sense: 5’- GAAGAACGUGUCAGCCTTGTCT -3’         |
|                  | Anti-sense: 5’- AAGACAGGACGGUCUUCCCT -3’       |
| PTTG1IP siRNA2   | Sense: 5’- GCUGAUAUCACAAUGUCCTT -3’           |
|                  | Anti-sense: 5’- CGBACACGGGUAAGUCAGTT -3’       |
| THRAP3 siRNA1    | Sense: 5’- GGGAUUCGAGGUGCAGTTT -3’            |
|                  | Anti-sense: 5’- UUGUGACCCUGGAAAUCCTT -3’       |
| THRAP3 siRNA2    | Sense: 5’- GCUGAUAACGCAAGCUUUTT -3’           |
|                  | Anti-sense: 5’- AAAGCUUGCUUCCUGAGCCT -3’       |

### Table S3. Probe sequences of RNA FISH and pull-down assay, Related to Figure 3.

| Target gene (Application) | Sequences (5’ to 3’) |
|--------------------------|----------------------|
| LINC00162 (FISH)         | GAATTTACCTCTTGGAAAGTCGCCAGGGAGTCTTCTGAGCGG |
| LINC00162 (pull-down) Primer F | TGAAGTGCTGAGGTTGAGGCAGC |
| LINC00162 (pull-down) Primer R | TTGATTTACCTGACTTTGTATATTTCCAGAAACA TTTT |
Figure S1. Analyze the potential functions of LINC00162 and silence and overexpression efficiency verification, Related to Figure 2.

(A) Molecular Function. (B) Cellular Component. (C) Biological Process. (D) Detection of the silencing and overexpression efficiency of LINC00162 in UM-UC-3 cells and T24 cells.
Figure S2. Construction of LINC00162 stable knockdown UM-UC-3 cell, Related to Figure 2.

(A) Structure of a stable knockdown plasmid vector. (B) Fluorescence picture of stable knockdown plasmid coated with lentivirus 6 hours after transfection in UM-UC-3 cells. (C) The silencing efficiency of the stable cell line that knocked down LINC00162 was detected by q-PCR.
Figure S3. Potential functional analysis of pull-down proteins and mass spectrum result of THRAP3, Related to Figure 5.

(A) Pathway analysis of pull-down sample proteins. (B and C) GO analysis of pull-down sample proteins.
(D) Mass spectrum of THRAP3 protein molecule
Transparent Methods

Tissue specimens and experimental animals

Thirty-two specimens of cancer and adjacent tissues from bladder cancer patients were irrespective of sex were collected from January 2018 to May 2019 in the urology department of the First Affiliated Hospital of Wenzhou Medical University. All patients underwent surgical resection and pathologically confirmed bladder cancer. Patients did not receive radiotherapy or chemotherapy before surgery. Patients were informed and signed informed consent before surgery. The research protocol was approved by the Wenzhou Medical Ethics Committee.

Four-week-old female athymic nude mice were purchased from Shanghai Slack Experimental Animal Center, and the experimental animal certificate number was SCXK (Su) 201904657. They were raised in the SPF-level experimental area of the Experimental Animal Center of Wenzhou Medical University. A total of 10 nude mice were used in the animal experiment of this study, and they were randomly divided into Vector and LINC00162 groups, with 5 nude mice in each group. All animal experiments were approved by the Animal Research Committee of Wenzhou Medical College, and animal research has been conducted in accordance with international guidelines.

Cell lines and main reagents

The human bladder cancer cell lines UM-UC-3 and T24, and the normal bladder epithelial cell line SV-HUC-1 were purchased from the ATCC cell bank. The qPCR kit was purchased from Promga, all qPCR primer sequences can be found in Table S1. TRIzol reagent was purchased from Invitrogen, and the GoScript™ Reverse Transcription System reagent was purchased from Promga.

LINC00162 overexpression vector (pcDNA3.1 (+/-)) was purchased from Guangzhou Bersinbio Company, PTTG1IP antibody was purchased from Abcam Company in the United States, THRAP3 antibody was purchased from Proteintech Company in the United States. Tubulin and GAPDH antibodies were purchased from Abway Company. Horseradish peroxidase (HRP)-labeled goat anti-mouse and rabbit IgG secondary antibodies were purchased from Cell Signaling Technology. The Luminescent Cell Viability Assay detection kit was purchased from Promega. Cell cycle detection kits were purchased from KGI. A RiboFECT CP transfection kit was purchased from
Guangzhou RiboBio Co., Ltd. CHIRP kits and RIP kits were purchased from the Guangzhou Bersinbio Company.

**Cell culture and transfection**

The human bladder cancer cell lines UM-UC-3 and T24 and the normal bladder epithelial cell line SV-HUC-1 were placed in complete medium supplemented with 10% fetal bovine serum, 100U/ml penicillin and 100μg/ml streptomycin, cultured in an incubator at 37°C and 5% CO₂ saturation humidity. Finally, a good and stable cell line was obtained for subsequent experiments. UM-UC-3 and T24 cell lines were transfected with riboFECT CP transfection reagent for siRNA or scramble according to the instructions, siRNAs sequence can be found in Table S2. After 48 h of transfection, the transfection efficiency was determined by q-PCR.

**ATP and EdU assay**

Cell Titer-Glo 2.0 Viability Assay detection kit was used to detect cell proliferation ability. Cells were seeded in 96-well plates and cultured in an incubator. Then cells were treated separately in groups as described in the text. 25μL of DDW and 25μl of ATP reagent were added to each well, plates were shaken in dark for five minutes and allowed to stand for ten minutes, and the fluorescence value was then measured with a microplate reader. The 5-ethynyl-2’-deoxyuridine (EdU) detection kit was used for cell proliferation detection according to the manufacturer’s instructions. The main process was as follows: The EdU solution was diluted with a complete cell culture medium at a ratio of 1,000:1 to prepare an appropriate amount of 50μM EdU medium. 100μl of 50μM EdU medium was added to each well and incubate for two hours. 100μL of cell fixation solution (4% paraformaldehyde in PBS) was added to each well and incubate for 30 minutes at room temperature. 2mg/mL glycine was added to each well and incubate for five minutes on a shaker. 100μl of 1X Apollo® Staining Reaction Solution was added to each well, and incubated for 30 minutes in a dark place at room temperature. Hoechst33342 reaction solution was diluted with deionized water at a ratio of 100:1, and store it in the dark. 100μL of 1X Hoechst 33342 reaction solution was added to each well. Plates were incubated for 30 minutes in dark at room temperature and shaken to remove the staining reaction solution. 100μL of PBS was added to each well and washed one to three times to elute Hoechst 33342 reaction solution. Images were acquired under a fluorescence microscope.
Annexin-V-FITC / PI staining flow cytometry

The cell cycle was detected on a flow cytometer using the Cycle Detection Kit (KeyGen Biotech). And the cell apoptosis was detected using cell apoptosis Kit (KeyGen Biotech). Both experiments were carried out in strict accordance with the instructions.

Western Blot (WB) assay

Cell lysis buffer (10mM Tris-HCl, pH 7.4, 1% SDS and 1mM Na$_3$VO$_4$) was used to prepare whole cell extracts, and proteins were extracted from cultured cells of each group. A BioDrop microanalyzer was used to determine protein concentration. Equal amounts of protein were separated by SDS-PAGE, transferred to a membrane, blocked, and the first antibody was added, and incubated at 4 °C overnight. On the next day, add goat anti-rabbit or mouse IgG second antibody coupled to alkaline phosphatase (AP) and incubate at 4 °C for three hours. A Typhoon FLA 7000 (GE, Pittsburgh, PA, USA) scanner was used to obtain images.

Nude mouse xenograft model

A vector of $3\times10^6$ cell density or LINC00162 shRNA UM-UC-3 stable cell line was inoculated subcutaneously in female athymic nude mice to construct a subcutaneous xenograft tumor model of nude mouse bladder cancer cells. Once the maximum tumor size reached 1,000 mm$^3$, the animals were sacrificed and the tumors were dissected for measurement.

Chromatin Isolation by RNA Purification (CHIRP) assay

Prepare cell suspension, lysate and sonicate according to the instructions of the ChIRP kit (Bersinbio, Guangzhou, China), and design a LINC00162 specific probe to attach to magnetic beads, probe sequence can be found in Table S3. The magnetic beads and cell lysate are incubated together to pull down the target RNA while enriching the DNA that interacts with it. The content of each gene in the product was detected by q-PCR.

RNA-FISH assay

Subcellular localization of LINC00162 was performed by FISH. Design a FISH-specific probe for LINC00162 (Sangon Biotech, Shanghai, China), probe sequence can be found in Table S3. Cells seeded on coverslips were fixed with 4%
paraformaldehyde for 20 minutes at room temperature and digested with proteinase K (Sangon Biotech) for 5 minutes at 37 °C. Next, the cells were fixed with 1% paraformaldehyde for 10 minutes and dehydrated in a gradient of 70%, 85%, and 100% alcohol. The probe-mixed solution was dropped on a coverslip, mounted and denatured at 73 °C for 3 minutes. In the dark, the hybridization lasted for 12-16 hours at 37 °C. Coverslips were counted with DAPI and fixed with anti-fluorescent attenuation reagent. The cells were examined and images were acquired under a confocal microscope (Leica, Mannheim, Germany).

**RNA Immunoprecipitation (RIP) assay**

Refer to the instructions of the RIP Kit (Bersinbio, Guangzhou, China) to treat the cells. Use THRAP3 antibody to capture the endogenous RNA-binding protein in the cytoplasm to prevent non-specific RNA binding. Immunoprecipitation separates the RNA-binding protein and its bound RNA together. q-PCR detects RNA fragments precipitated by RIP.

**RNA pull-down assay**

The RNA-specific probes designed for LINC00162 were attached to magnetic beads and the cells were lysed according to the instructions of the RNA pull-down Kit (Bersinbio, Guangzhou, China). The magnetic beads were incubated with the cell lysate, and the protein interacting with LINC00162 was pulled. The pull down product was analyzed by mass spectrometry.

**Immunohistochemistry (IHC)**

Nude mouse transplanted tumor tissue was embedded with hard wax, sliced, dewaxed and rehydrated. After incubation with primary and secondary antibodies, it was counterstained with hematoxylin (Solarbio, China). Images were taken with microscopes at 100x and 400x magnification (Leica, Mannheim, Germany). IHC antibody: PTTG1IP (Abcam, USA).

**Statistical analysis**

GraphPad Prism 6 software was used for statistical analysis. Comparisons between groups were performed using two independent sample t tests. The difference was statistically significant at P <0.05. And data are represented as mean ± standard deviation (SD).