# LncRNA *LINC00969* modified by *METTL3* attenuates papillary thyroid cancer progression in an m$^6$A-dependent manner

Chaogang Huang[1,B,D,F], Ziqi Duan[2,A,F], Baojie Chen[1,C,F], Hailiang Xia[1,E,F], Guangxin Wang[1,E,F]

[1] Department of General Surgery, Wuhan Third Hospital (Tongren Hospital of Wuhan University), China
[2] Department of General Surgery, Jianghan University, Wuhan, China

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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**Address for correspondence**
Guangxin Wang
E-mail: wangguangxin0627@163.com

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## Abstract

**Background.** The long non-coding RNA (lncRNA) *LINC00969* is involved in human disease progression, and n6-methyladenosine (m$^6$A) modification of lncRNAs in cancer has been proven to be a key regulatory mechanism. However, our understanding of its effects and mechanisms of action in papillary thyroid carcinoma (PTC) remains limited.

**Objectives.** This study aimed to elucidate the role of methyltransferase-like 3 (METTL3)-induced m$^6$A modification of *LINC00969* in PTC tumorigenesis.

**Materials and methods.** Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed to analyze *LINC00969* and *METTL3* mRNA levels in PTC. The regulation of *LINC00969* by METTL3 was confirmed using cell function experiments, molecular biology assays and bioinformatics analysis. *LINC00969* stabilization analysis was performed to verify the regulatory roles of *METTL3* and *LINC00969*.

**Results.** *LINC00969* expression was downregulated in PTC tissues. Increased *LINC00969* expression inhibited the invasion, growth and migration of PTC cells. *METTL3* downregulation in PTC mediated the m$^6$A modification of *LINC00969*, increasing its stability. Furthermore, *METTL3* levels were downregulated in PTC, and its silencing partially reversed the inhibitory effect of *LINC00969* overexpression on PTC cell malignancy.

**Conclusions.** *LINC00969* overexpression inhibits PTC cell malignancy via *METTL3*-mediated m$^6$A modification. These findings suggest that *METTL3-m$^6$A-LINC00969* is a promising therapeutic target for PTC.

**Key words:** papillary thyroid cancer, long non-coding RNA, *METTL3*, m$^6$A, *LINC00969*

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# Background

The incidence of thyroid cancer (THCA), the most common endocrine malignancy, has increased over the past 3 decades.[1] Papillary thyroid carcinoma (PTC), the most common subtype of THCA, accounts for 80–85% of all THCA cases.[2] Papillary thyroid carcinoma has been documented to exhibit a favorable prognosis, with a 5-year survival rate approaching 100%.[3] However, some cases of locally infiltrating PTC have a high recurrence rate.[4] In recent years, targeted therapies, particularly small-molecule therapies targeting receptor tyrosine kinases, have been evaluated in clinical trials to improve the survival of patients with PTC presenting with local infiltration.[2,5] Nevertheless, there is a need for further investigation into more valuable molecular targets and mechanisms for the clinical management of PTC.

With the advancement of RNA sequencing technology, scientists are increasingly focusing on a specific class of RNA molecules, namely long non-coding RNAs (lncRNAs), which are over 200 nucleotides in length and do not encode proteins.[6] The lncRNAs play crucial roles in diverse biological processes by acting as regulators that interact with RNA, DNA and proteins.[7,8] Studies have shown that lncRNAs regulate carcinogenesis by participating in the invasion, metastasis, differentiation, proliferation, and metabolic reprogramming of tumor cells.[9] In PTC, a wide range of lncRNAs are involved in tumor occurrence, including lncRNA GAS8-AS1,[10] LINC00671[11] and lncRNA ABHD11-AS1.[12] *LINC00969*, also known as the *MIR570* host gene, plays inconsistent roles in various human diseases. For instance, *LINC00969* accelerates intervertebral disc degeneration by enhancing apoptosis in the nucleus pulposus,[13] acts as a potential biomarker for the development of type 2 diabetes mellitus complicated by carotid atherosclerotic plaques,[14] and promotes gefitinib resistance in lung cancer cells.[15] These studies demonstrate that LINC00969 epigenetically regulates multiple signaling pathways and that epigenetic regulation plays an important role in many biochemical processes associated with human diseases.[16] However, the effect of LINC00969 on PTC remains uncertain and requires further investigation.

N6-methyladenosine ($m^6A$) is a class of dynamically reversible RNA modifications in eukaryotic mRNAs that cluster around the stop codon and 3' untranslated region.[17] N6-methyladenosine regulates various cellular pathways and processes by regulating mRNA splicing, expression, decay, and translation.[18] There is growing evidence of a potential link between *m6A* and cancer, with changes in *m6A* levels affecting the progression or remission of tumors, including lung,[19] liver,[20] ovarian,[21] and breast[22] cancers. Methyltransferase-like 3 (METTL3), possessing methyltransferase activity, regulates the progression of multiple tumors, including PTC.[23] For example, *METTL3* is reported to be downregulated in PTC and to positively regulate STEAP2 levels by mediating *m6A* modification, which in turn inhibits PTC cell malignancy.[24,25] However, the mechanisms through which *LINC00969* and *METTL3* affect PTC require further investigation.

# Objectives

This study aimed to elucidate the regulatory role of *LINC00969* in PTC development and to demonstrate the tumor-suppressive role of *METTL3* in PTC via $m^6A$ modification. The findings of our study broaden our understanding of the mechanisms underlying PTC development and provide valuable targets for PTC therapy.

# Materials and methods

## Clinical specimens

Samples from 32 patients diagnosed with PTC at Wuhan Third Hospital (Wuhan, China), including both PTC and corresponding normal tissues, were collected by surgical excision. The inclusion criteria were as follows: 1) all patients were initially diagnosed with PTC by 2 pathologists and 2) none of the patients underwent any treatment before surgery. The exclusion criteria were as follows: 1) patients who did not provide written consent and 2) patients with comorbidities such as thyroiditis and cervical lymphadenopathy. The clinical characteristics of patients with PTC is listed in Table 1. All clinical specimens were stored in a –80°C refrigerator. Prior to sample collection, all participants signed an informed consent form. The collection and use of human specimens were approved by the Ethics Committee of the Wuhan Third Hospital (approval No. KY2023-012).

**Table 1.** Clinical characteristics of papillary thyroid cancer patients (n = 32)

| Variables | | n (%) |
|---|---|---|
| Age [years] | <45 | 18 (56.3) |
| | ≥45 | 14 (43.7) |
| Sex | male | 10 (31.3) |
| | female | 22 (68.7) |
| Multifocality | yes | 11 (34.4) |
| | no | 21 (65.6) |
| Location | left lobe | 13 (40.6) |
| | right lobe | 16 (50.0) |
| | bilateral | 2 (6.3) |
| | isthmus | 1 (3.1) |
| Tumor stage | I–II | 20 (62.5) |
| | III–IV | 12 (37.5) |
| Lymph node metastasis | yes | 17 (53.1) |
| | no | 15 (46.9) |

## Cell culture

The normal human thyroid cell line Nthy-ori-3-2 (BNCC340487) and PTC cell lines, including TPC-1 (BNCC337912) and IHH-4 (BNCC360242), were obtained from the BeNa Culture Collection (BNCC, Beijing, China). IHH-4 and TPC-1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; BNCC), whereas Nthy-ori-3-1 cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 (BNCC). Cell supernatants were supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin and maintained at 37°C with 5% $CO_2$.

## Cell transfection

*LINC00969* or *METTL3* sequences were cloned into a pcDNA3.1 vector for overexpression of *LINC00969* or *METTL3* (LINC00969-OE or METTL3-OE; RiboBio, Wuhan, China), with blank pcDNA3.1 used as the negative control (NC-OE). In addition, RiboBio synthesized the small interfering RNAs (siRNAs) targeting METTL3 (si-METTL3) and si-NC. Transfection of vector (2 μg/mL) or oligonucleotide (50 nM) was performed using Lipo6000 reagent (Beyotime Biotechnology, Shanghai, China).

## qRT-PCR

A TransZol Up Plus RNA kit (TransGen Biotech, Beijing, China) was used to extract total RNA. Reverse transcription and quantitative reverse transcription polymerase chain reaction (qRT-PCR) were performed using a cDNA Synthesis Kit (Fermentas, Waltham, USA) and SYBR Green Master Mix (Takara, Shiga, Tokyo, Japan), respectively. The qRT-PCR conditions were as follows: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. Relative expression was determined using the $2^{-\Delta\Delta Ct}$ method with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the internal reference. Primer sequences are shown in Table 2.

Table 2. Primer sequences of *LINC00969* and *METTL3* in qualitative reverse transcription polymerase chain reaction (qRT-PCR) assay

| Name | Primer sequences |
|---|---|
| *LINC00969* | Forward (5′-3′): TGGTGGAAGTGAGACCGAAAT |
| | Reverse (5′-3′): GTGATCCGTCCCAAGACAGC |
| *METTL3* | Forward (5′-3′): CAAGCTGCACTTCAGACGAA |
| | Reverse (5′-3′): GCTTGGCGTGTGGTCTTT |
| *GAPDH* | Forward (5′-3′): GAAGGTGAAGGTCGGAGTC |
| | Reverse (5′-3′): GAAGATGGTGATGGGATTTC |

## Nucleus and cytoplasm localization

RNA from the cytoplasm and nuclei of IHH-4 and TPC-1 cells was isolated using a Nuclear and Cytoplasmic RNA Purification Kit (Norgen, Thorold, Canada) as described in a previous study. Quantification of LINC00969 levels in the cytoplasm and nucleus was performed using qRT-PCR.[26]

## CCK-8 analysis

Cells ($3 \times 10^3$ cells/well) were seeded into 96-well plates, and 100 μL of DMEM medium supplemented with 10% FBS was added. Cells were incubated for 0, 24, 48, and 72 h. Each well was then supplemented with Cell Counting Kit-8 (CCK-8) reagent (10 μL; Beyotime Biotechnology), and the plates were incubated at 37°C for 1 h. Optical density at 450 nm ($OD_{450}$) was measured using a microplate reader (model 1681130; Bio-Rad, Hercules, USA).

## Colony formation assay

Papillary thyroid carcinoma cells were collected, adjusted to a concentration of $2 \times 10^3$/mL, and transferred to the corresponding 6-well plates at 1 mL per well. Once colonies had grown to the appropriate size (after approx. 14 days of cell culture), the supernatant was removed, and the cells were washed. Paraformaldehyde (4%) was added for fixation at 25°C for 10 min. Subsequently, 700 μL of crystal violet staining solution was added for a 5-min staining period. The plates were allowed to dry, and images were captured using an optical microscope (model GX53; Olympus Corp., Tokyo, Japan). The number of clones in each well was determined.

## Transwell assay

Dulbecco's modified Eagle's medium (500 μL) containing IHH-4 and TPC-1 cells ($2 \times 10^5$) was added to the upper transwell insert, with or without Matrigel pre-addition for invasion or migration assays. The prepared inserts were then placed in the lower compartment with 750 μL of complete DMEM medium containing 10% FBS. Invading or migrating cells were stained with 0.1% crystal violet after 24 h of incubation. Cell counts were recorded under an optical microscope (model GX53; Olympus Corp.).

## Methylated RNA immunoprecipitation assay

Methylated RNA immunoprecipitation (MeRIP) assays were conducted using the Magna MeRIP $m^6A$ kit purchased from Cloud-Seq Biotech (Shanghai, China). Total RNA (150 μg) extracted from cells treated with METTL3-OE or negative control of overexpression vector (NC-OE) was fragmented to produce fragments of 100 nucleotides or smaller. The fragmented RNA was then immunoprecipitated using magnetic beads precoated with 3 μg of anti-$m^6A$ antibody or anti-IgG. Finally, the immunoprecipitated RNA fragments were analyzed using qRT-PCR.[27]

## RNA stabilization assay

IHH-4 and TPC-1 cells were treated with 2 μg/mL of the transcriptional inhibitor actinomycin D for 0, 3 and 6 h to block transcription. The cells were collected for RNA extraction, and qRT-PCR was performed to measure the LINC00969 levels.

## Statistical analyses

Data expressed as mean ± standard deviation (±SD) were analyzed using GraphPad Prism v. 9 software (GraphPad, San Diego, USA). The normality of all data was assessed using the Shapiro–Wilk test (Supplementary Table 1), and the homogeneity of variance was analyzed using either the F test or Brown–Forsythe test (Supplementary Table 2). If the data followed a normal distribution and exhibited heterogeneous variance, Student's t-test was used to compare differences between 2 groups, with significance set at $p < 0.05$. If the data did not follow a normal distribution or heterogeneous variance or if the sample size per group was less than 20, the Mann–Whitney U test was used for 2 groups, and the Kruskal–Wallis test followed by Dunnett's post hoc test was used for multiple groups, with significance set at $p < 0.05$ (Supplementary Table 3). In addition, the data from Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/index.html) were analyzed using the R package limma.

# Results

## *LINC00969* exhibits low expression in PTC

Gene Expression Profiling Interactive Analysis is an online tool that analyzes differentially expressed lncRNAs in THCA samples. Using GEPIA, we found that *LINC00969* expression was significantly lower in 512 THCA samples compared to 337 normal samples (Fig. 1A). To confirm this

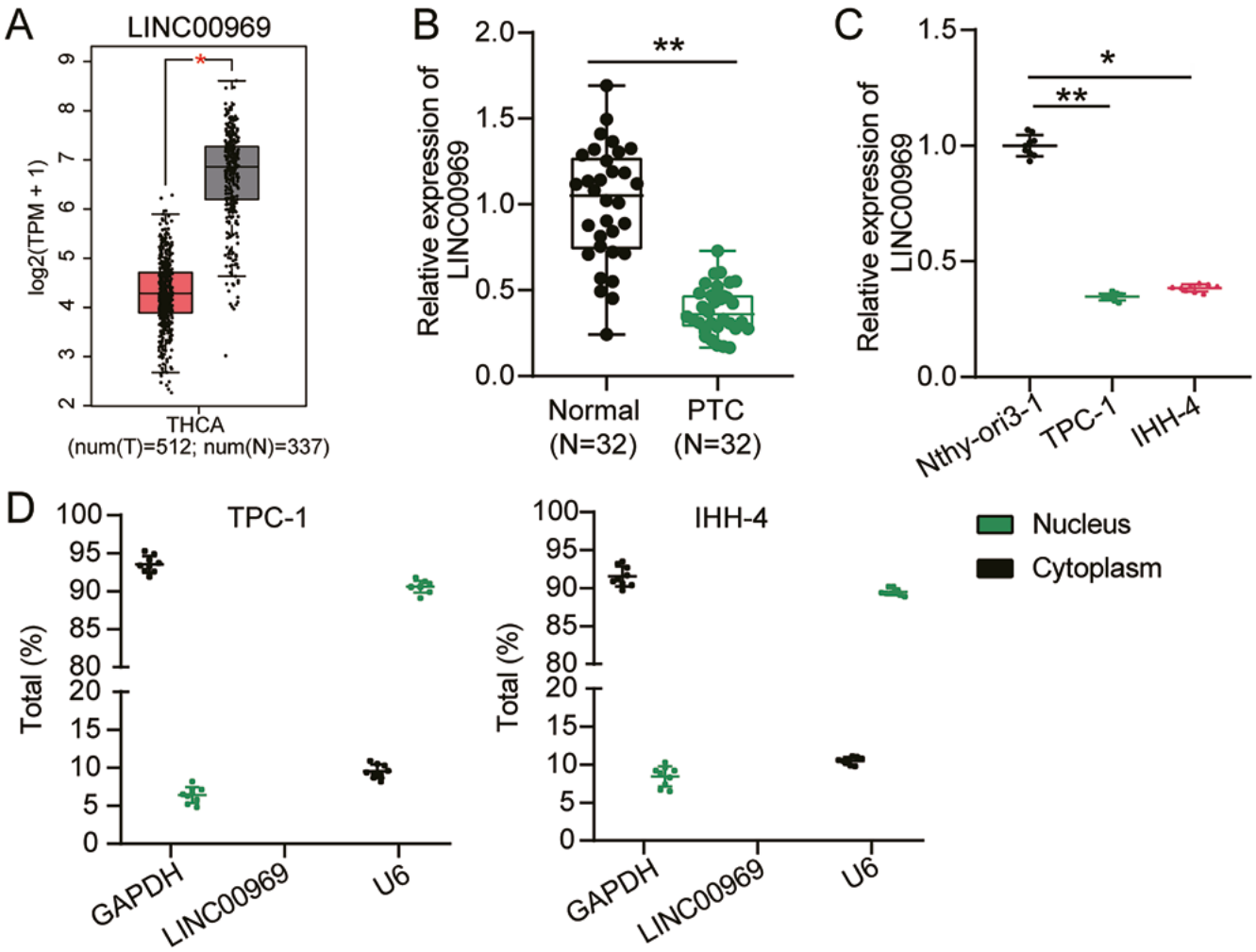


Fig. 1. *LINC00969* exhibits low expression in papillary thyroid carcinoma (PTC). A. Gene Expression Profiling Interactive Analysis (GEPIA) showed the *LINC00969* expression in thyroid cancer (THCA); *p < 0.001; B. *LINC00969* expression in PTC and normal tissues was analyzed using qualitative reverse transcription polymerase chain reaction (qRT-PCR); **p < 0.001; C. *LINC00969* expression in Nthy-ori-3-1 and PTC cell lines (IHH-4 and TPC-1) was analyzed using qRT-PCR; *p < 0.05, **p < 0.001 vs Nthy-ori-3-1; D. *LINC00969* expression in the nucleus and cytoplasm of PTC cells was analyzed using qRT-PCR

finding, we collected PTC tissues and adjacent normal tissues from 32 patients with PTC and assessed *LINC00969* expression using qRT-PCR. The results showed a 70% downregulation of *LINC00969* expression in PTC tissues (Fig. 1B). Subsequently, we analyzed *LINC00969* expression in 2 PTC cell lines (TPC-1 and IHH-4) and the normal human thyroid cell line Nthy-ori-3-2 using qRT-PCR, and *LINC00969* was found to be downregulated in the PTC cell lines (Fig. 1C). Furthermore, a nuclear–cytoplasmic isolation assay was conducted to determine the subcellular localization of LINC00969, showing higher levels of LINC00969 in the cytoplasm of PTC cells than in the nucleus, suggesting that LINC00969 was enriched in the cytoplasm (Fig. 1D). Taken together, these findings suggest that LINC00969 expression was enriched in the cytoplasm and downregulated in PTC.

## *LINC00969* overexpression inhibits PTC cell malignancy

To confirm the function of *LINC00969* in PTC, we constructed the *LINC00969* overexpression vector (LINC00969-OE) and its negative control (NC-OE), which were then transfected into PTC cells. Subsequently, qRT-PCR was used to detect *LINC00969* expression and verify the transfection efficiency. The results revealed a 6-fold increase in *LINC00969* expression in the LINC00969-OE group compared to the NC-OE group, indicating the high transfection efficiency of LINC00969-OE (Fig. 2A). Cell function assays were performed to analyze the effects of LINC00969 on the proliferation, migration and invasion of PTC cells. The CCK-8 assay demonstrated an approx. 40% reduction in cell viability in the LINC00969-OE group (Fig. 2B). Colony formation analysis revealed that the number of colonies in the LINC00969-OE group decreased by nearly 70% compared to the NC-OE group (Fig. 2C). Transwell assay results showed that *LINC00969* upregulation led to approx. 62% and 70% reductions in PTC cell migration and invasion, respectively (Fig. 2D,E). These results demonstrate that *LINC00969* overexpression inhibits PTC cell malignancy.

## *METTL3* exhibits low expression in PTC and mediates the m$^6$A modification of LINC00969

To identify the molecular targets of m$^6$A in PTC, we determined the m$^6$A modification site of *LINC00969* using RMBase v2.0 (Fig. 3A). Using the GEPIA database, we found a positive correlation between *LINC00969* and *METTL3* expression in THCA, and found that *METTL3* expression was reduced in THCA (Fig. 3B,C). In our clinical samples from 32 patients with PTC, qRT-PCR analysis also showed low METTL3 levels in PTC tissues and a positive correlation with *LINC00969* expression (Fig. 3D,E). The m$^6$A modification of LINC00969 by METTL3 was examined using the MeRIP assay, and the results revealed that the m$^6$A levels of LINC00969 were significantly increased following *METTL3* upregulation, indicating that METTL3 could regulate *LINC00969* through m$^6$A modification in PTC (Fig. 3F). Moreover, RNA stabilization assays revealed that *METTL3* overexpression increased the resistance of LINC00969 to actinomycin D, indicating that *METTL3* overexpression stabilized the RNA structure of *LINC00969* (Fig. 3G). Therefore, *METTL3* downregulation in PTC could mediate the m$^6$A modification of LINC00969, thereby stabilizing its RNA structure.

## *METTL3* silencing partially reverses the malignant blockage of PTC cells caused by *LINC00969* overexpression

Subsequently, we investigated the effects of METTL3 combined with LINC00969 on the biological functions of PTC cells by transfecting the LINC00969-OE vector and si-METTL3 into PTC cells. The results of CCK-8 and colony formation assays showed that the decreased proliferation of PTC cells caused by *LINC00969* overexpression was increased further when LINC00969-OE and si-METTL3 were co-transfected into PTC cells (Fig. 4A,B). Moreover, transwell analysis revealed that both migration and invasion of PTC cells were impaired by the transfection of LINC00969-OE into PTC cells; however, this inhibitory effect on migration and invasion was enhanced by co-transfection of LINC00969-OE and si-METTL3 into PTC cells (Fig. 4C,D). In conclusion, *METTL3* knockdown reverses the inhibitory effects of *LINC00969* overexpression on PTC malignancy.

# Discussion

In this study, we elucidated the impact of *LINC00969* on PTC cell function via the m$^6$A modification of METTL3. We found that *LINC00969* was downregulated in PTC, and its upregulation contributed to PTC cell malignancy, thereby alleviating PTC tumorigenesis. This study also confirmed that the m$^6$A modification of *LINC00969* could be induced by *METTL3*, thus enhancing the stability of *LINC00969*. *METTL3* has been shown to suppress PTC progression by mediating the m$^6$A modification of LINC00969.

Studies have found that dysregulated lncRNAs hold great potential as biomarkers of PTC. For instance, a study published in 2021 confirmed that *LINC00671* attenuates PTC cell malignancy by interacting with LDHA and STAT3.[11] In 2022, another lncRNA, ABHD11-AS1, was shown to enhance PTC cell malignancy by interacting with the EPS15L1/EGFR signaling pathway.[12] These studies revealed the diverse functions of different lncRNAs in PTC. Here, we found that *LINC00969* was downregulated

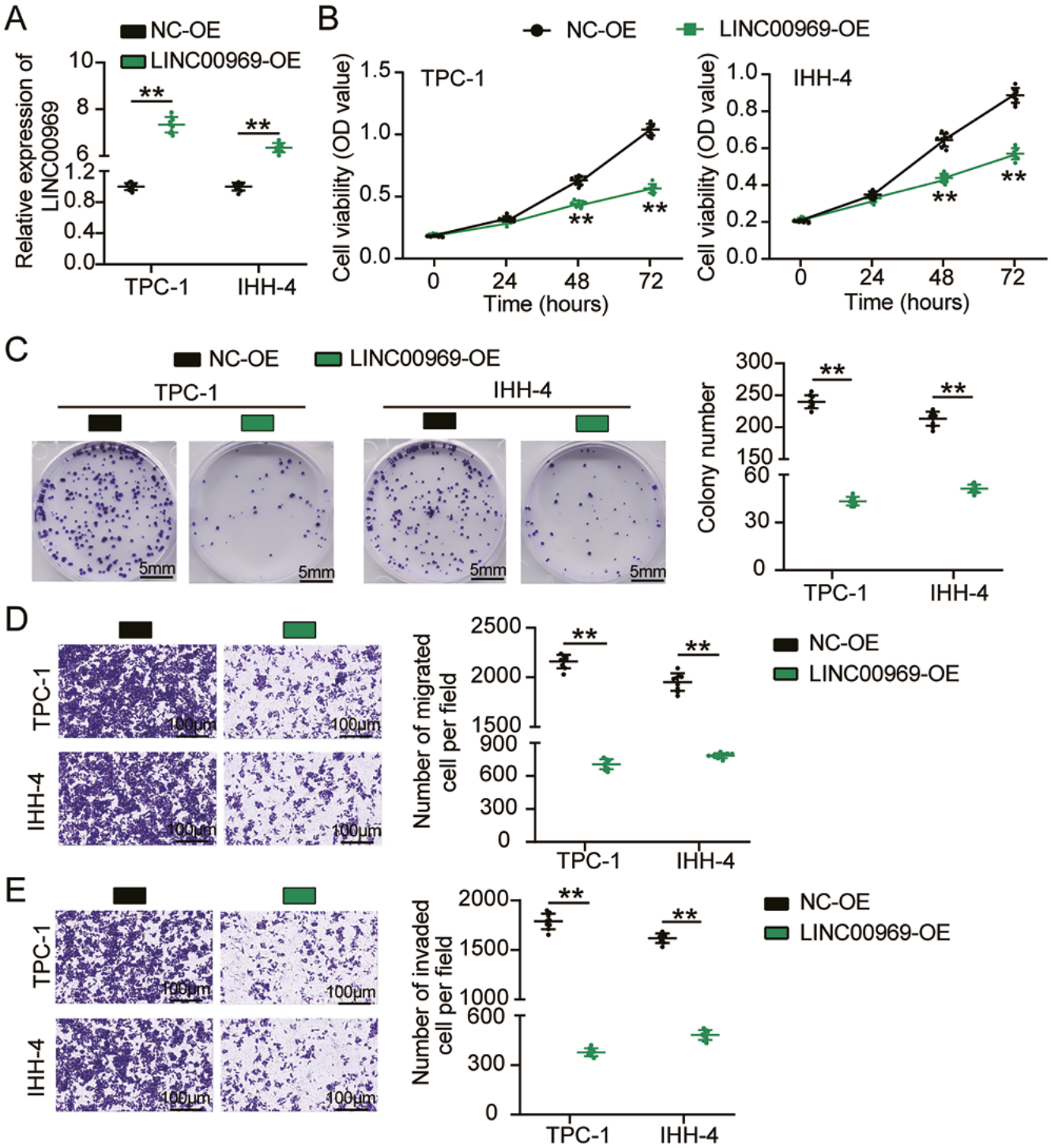


Fig. 2. Overexpression of *LINC00969* inhibits papillary thyroid carcinoma (PTC) cell malignancy. A. Qualitative reverse transcription polymerase chain reaction (qRT-PCR) revealed the *LINC00969* expression in PTC cells transfected with LINC00969-OE or negative control of overexpression vector (NC-OE); B,C. Cell Counting Kit-8 (CCK-8) and colony formation assays detected PTC cell proliferation following transfection with LINC00969-OE or NC-OE; D,E. Transwell assay detected the migration and invasion of PTC cells transfected with LINC00969-OE or NC-OE; **p < 0.001 vs NC-OE

OD – optical density.

in PTC. After reviewing the literature, only Dai et al.[15] explored the function of *LINC00969* in lung cancer, proving that *LINC00969* promotes lung cancer progression by inhibiting cancer cell apoptosis. In contrast to the findings of Dai et al., we found that LINC00969 repressed PTC cell survival and metastasis, suggesting that LINC00969 inhibited PTC progression. This is the first study to demonstrate that *LINC00969* plays different roles in different cancer types. LINC00969 is an antitumor factor in PTC and an oncogenic factor in lung cancer.

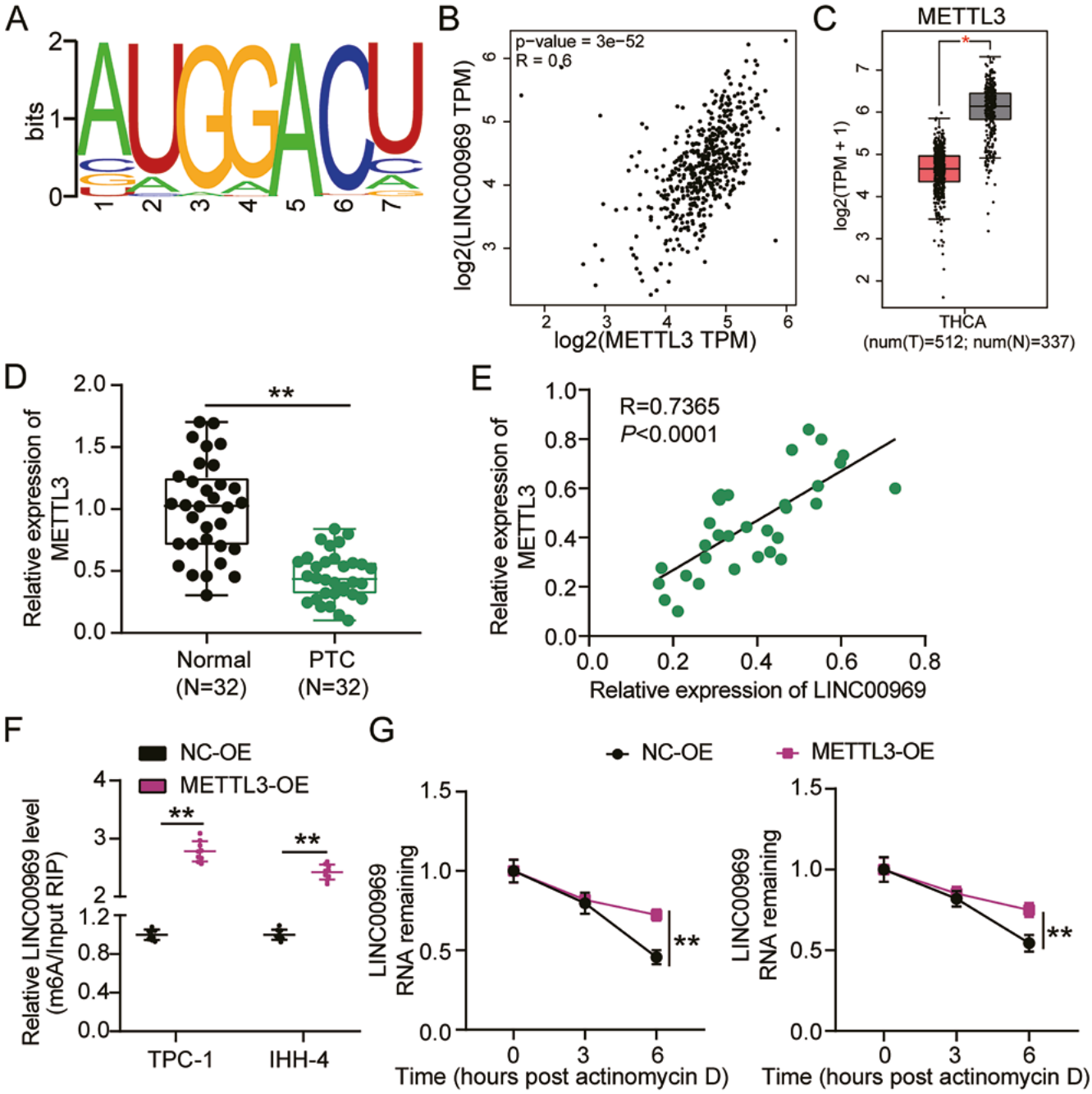


Fig. 3. *METTL3* exhibits low expression in papillary thyroid carcinoma (PTC) and mediates the m$^6$A modification of *LINC00969*. A. m$^6$A modification site of *LINC00969* according to RMBase v 2.0; B. Gene Expression Profiling Interactive Analysis (GEPIA) showed the correlation between *LINC00969* and *METTL3* in thyroid cancer (THCA); C. *METTL3* expression in THCA using GEPIA; D. Qualitative reverse transcription polymerase chain reaction (qRT-PCR) revealed the *METTL3* expression in PTC and normal tissues; **p < 0.001; E. Pearson's analysis was used to assess the correlation between *LINC00969* and *METTL3* in PTC samples; F. MeRIP assay detected the m$^6$A modification of *LINC00969* in transfected PTC cells; **p < 0.001 vs NC-OE; G. *LINC00969* stability in transfected NPC cells; **p < 0.001

The m$^6$A modifications that regulate lncRNAs have been recognized.[17] Therefore, we investigated the m$^6$A modification of LINC00969 in PTC. Our research showed that METTL3 in PTC mediates the m$^6$A modification of LINC00969. The literature suggests that *METTL3* regulates lncRNAs to affect cancer progression. For instance, in lung cancer, METTL3 induces m$^6$A modifications that enhance the stability of ABHD11-AS1 transcripts and are strongly associated with unfavorable patient prognosis.[28] Likewise, in prostate cancer, *METTL3* regulates the m$^6$A modification of the lncRNA *SNHG7*, enhancing its stability and subsequently accelerating cancer glycolysis.[29] Furthermore, in gastric cancer, METTL3 induces the m$^6$A modification of lncRNA THAP7-AS1 through an IGF2BP1-dependent pathway, which accelerates tumor development.[30] Previous studies have confirmed the oncogenic role of *METTL3* in cancer. However, our study is the first to provide evidence that *METTL3* is an antitumor factor

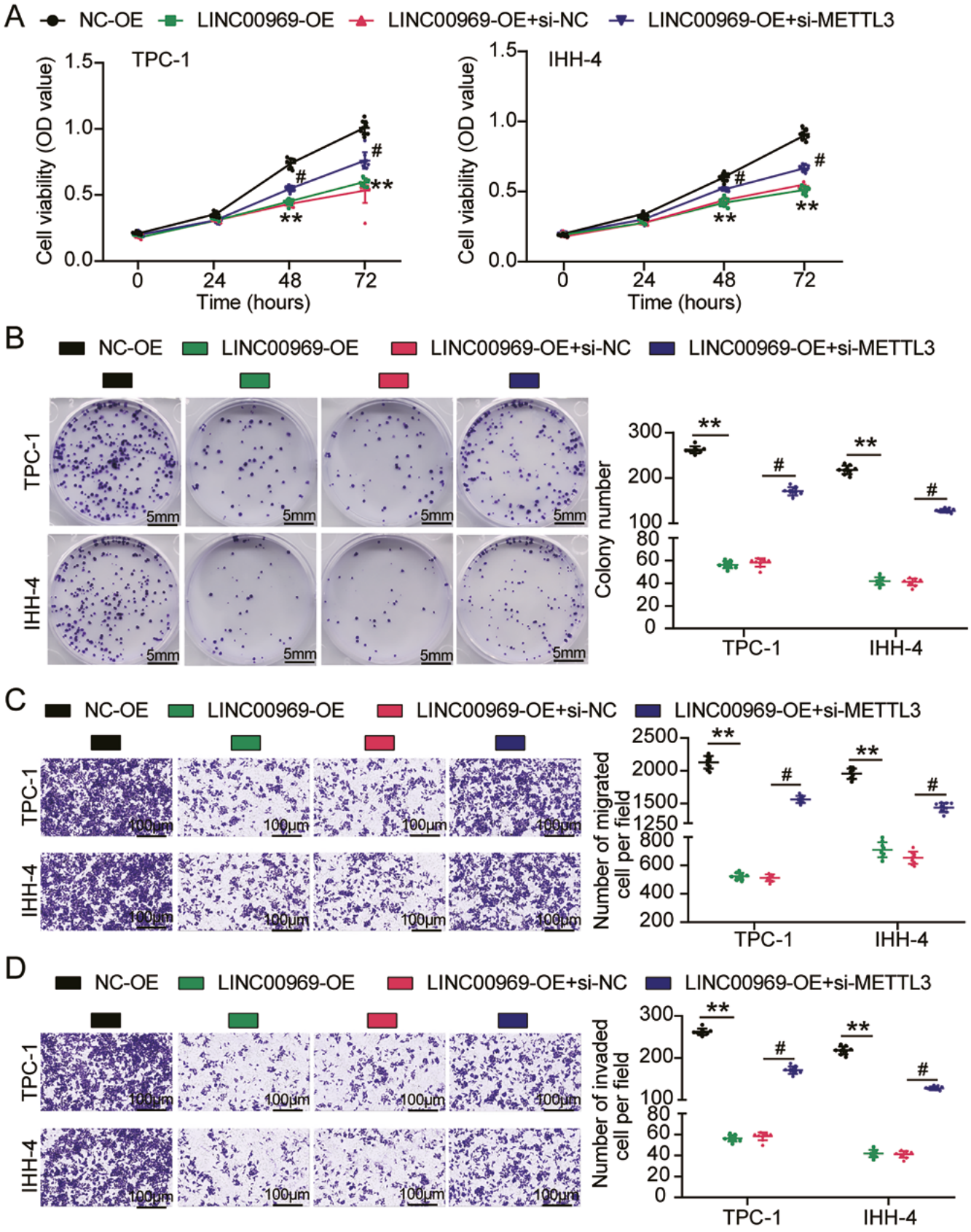


Fig. 4. Silencing of *METTL3* partially reverses the malignancy of papillary thyroid carcinoma (PTC) cells caused by *LINC00969* overexpression. A,B. Cell proliferation in transfected PTC cells was revealed using Cell Counting Kit-8 (CCK-8) and colony formation assay; C,D. Cell migration and invasion in transfected PTC cells were detected using transwell assay; **p < 0.001 vs negative control of overexpression vector (NC-OE); #p < 0.05 vs *LINC00969*-OE + negative control of siRNA (si+NC); OD – optical density.

in PTC, showing that its downregulation in PTC and silencing promote PTC cell malignancy. Further analysis of the inhibitory effect of *METTL3* on PTC revealed that METTL3 mediated the m$^6$A modification of LINC00969 to enhance *LINC00969* expression, thereby exerting an inhibitory effect on PTC cell malignancy. Our results align with the conventional understanding of *METTL3* as a carcinogenic factor in cancer and that the different roles and regulatory mechanisms of *METTL3* in different cancers need to be explored.

## Limitations

This study elucidated the regulatory mechanism of LINC00969 interaction with METTL3 in PTC, but there were some limitations. First, the m$^6$A modification consists of a "reader" protein, an "eraser" enzyme and a "writer" enzyme, which all play a role in regulating tumor cell malignancy in PCT.[31] Although our study confirmed that the "writer" enzyme METTL3 mediates m$^6$A modification of LINC00969, we have not investigated the "reader" protein or "eraser" enzyme. Therefore, further exploration is needed to understand the roles of LINC00969 and other m$^6$A-modified proteins in PTC. Furthermore, our study collected clinical samples from only 32 patients with PTC, resulting in a small sample size that limited our ability to ascertain the clinical significance of LINC00969 and METTL3 in PTC. Future studies should aim to collect more extensive clinical samples to explore the relevance of LINC00969 and METTL3 to the histopathological phenotypes. Furthermore, the statistical analysis in our study was exploratory and did not incorporate multiple comparisons correction. Consequently, caution is warranted in interpreting the results due to the potential for uncontrolled type I error probability, which may also impact the validity of future meta-analyses. Nevertheless, this exploratory analysis provides a foundation for further research in the field.

# Conclusions

This study revealed the downregulation of *LINC00969* and *METTL3* in PTC tissues and cells. Upregulation of *LINC00969* inhibited PTC proliferation, migration and invasion; however, silencing *METTL3* reversed the inhibitory effects of *LINC00969* on PTC malignancy. Further mechanistic analysis confirmed that *METTL3* mediated the m$^6$A modification of *LINC00969*, stabilizing its RNA structure in PTC cells. This study presents a novel PTC regulatory mechanism based on the *METTL3/LINC00969* axis, which may provide new diagnostic or therapeutic biomarkers for PTC.

## Supplementary data

The Supplementary materials are available at https://doi.org/10.5281/zenodo.11080300. The package includes the following files:

Supplementary Table 1. The results of normality test.

Supplementary Table 2. The results of homogeneity of variance.

Supplementary Table 3. The results of statistical analysis.

## Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Consent for publication

Not applicable.


### ORCID iDs

Chaogang Huang https://orcid.org/0009-0002-1589-7933
Ziqi Duan https://orcid.org/0009-0007-8720-8697
Baojie Chen https://orcid.org/0009-0006-3795-6227
Hailiang Xia https://orcid.org/0009-0001-6814-2756
Guangxin Wang https://orcid.org/0009-0009-4981-3460


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