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The Aurora-A/NPAT/Wnt-β-catenin pathway enhances the metastasis and stemness of lung cancer cells

Y. Zhao[#*], Q. Chen[#], P. Li, X. Fu, J. Duan, Y. Tang, Y. Ma, Q. Zhou[*]

Department of Medical Oncology, Huazhong University of Science and Technology Union Shenzhen Hospital, Shenzhen, Guangdong 518052, China

► ***Original article***

Corresponding authors:
Yan Zhao, Ph.D.,
Qiming Zhou, M.D.,
E-mail:
zhaoyan_nsyy@163.com,
zqm961221@163.com

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# These authors contributed equally to this work.

ABSTRACT

Background: Aurora-A kinase (Aurora-A) serves as an oncogene in many cancers, but whether its expression is linked to the stemness of lung cancer (LC) cells remains obscure. Hence, this study probed the impact as well as mechanism of Aurora-A in affecting the stemness of LC cells. ***Materials and Methods:*** Functional assays were implemented to assess the malignant behaviors along with stemness of LC cells. Western blot analysis detected the stemness markers together with the Wnt-β-catenin pathway-linked genes protein levels. TOP/FOP-Flash reporter assay assessed the activity of the Wnt/β-catenin pathway. Hematoxylin and eosin staining as well as immunohistochemistry analysis of tumor tissues were implemented. ***Results:*** Our findings indicated that Aurora-A was up-regulated in LC cells, and silenced Aurora-A hindered LC cell proliferation, migration, invasion along with stemness. Nuclear protein, coactivator of histone transcription (NPAT) was also high-expressed in LC cells, and was positively modulated by Aurora-A. Silenced NPAT inhibited LC cell malignant behaviors along with stemness. Aurora-A activated the Wnt/β-catenin pathway and promoted LC cell malignant behaviors along with stemness via regulating NPAT. In an established xenograft model, Aurora-A inhibition reduced tumor growth, metastasis along with stemness in vivo. ***Conclusion:*** In summary, Aurora-A/NPAT/Wnt-β-catenin signaling pathway accelerates LC cell malignant behaviors along with stemness. The modulation of Aurora-A might be an underlying therapeutic approach in LC patients.

INTRODUCTION

In recent years, the occurrence of lung cancer (LC) in China is increasing year after year, and the age of onset is becoming younger and younger [1]. Due to the high heterogeneity of LC, the curative effect of different types of LC varies greatly [2]. On the whole, it has the characteristics of high invasiveness, high metastasis, short recurrence time, poor prognosis, and low overall survival rate [3]. Currently, there is still a lack of effective therapy for poorly differentiated LC [4]. To elucidate the signal pathway of repressing the proliferation as well as metastasis of LC, corresponding therapeutic measures can be taken to make the tumor cells go towards differentiation or apoptosis and achieve the purpose of curing the tumor [5].

The process by which malignant tumor cells proliferate and detach from the primary site is the initial stage of metastasis [6]. Numerous studies have concentrated on the key cell side populations that promote proliferation and metastasis initiation, with the cancer stem cell (CSC) theory as the mainstream [7]. CSC is characterized by self-renewal and infinite proliferation, and is a type of the decisive factors in tumor occurrence, development, drug resistance, and metastasis [8]. The proliferation and metastasis ability of tumor cells with high expression of CD133, CD44, OCT4, PGE2, and other stemness-related markers such as Oct4 and Sox2 are increased significantly [9]. LC stem cells (LCSC) enable tumor tissues to have the capacity of self-renewal, multi-direction differentiation, along with infinite proliferation, which is a critical factor in tumor metastasis and recurrence [10]. Whether the tumor stem cells can be completely eliminated becomes an important prerequisite for tumor remission or even cure [11]. Elucidation of the regulatory mechanism of repressing the stemness of LC cells is of great guiding significance for clinical treatment.

Aurora-A kinase (Aurora-A) is a part of the Aurora kinase family, which is an important family of silk/threonine protein kinases implicated in modulating cell mitosis [12]. Aurora-A is high-expressed in many human tumor tissues and has a crucial role in promoting tumor progression [13]. A recent high-throughput data analysis has shown that Aurora-A has a crucial potential in controlling the self-renewal as well as pluripotent capacity of embryonic stem cells (ESCs) [14]. Overexpressed Aurora-A reduces *p53* gene expression and its downstream genes through phosphorylation, thus maintaining the

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undifferentiated state of ESCs. The deletion of Aurora -A causes the activation of *p53* gene, and then inhibits embryonic stems-linked genes expression such as *OCT4*, *Nanog*, *Sox2*, leading to reduced cell self-renewal ability as well as the terminal differentiation of cells. Aurora-A can mediate resistance to chemotherapy and radiotherapy and participate in tumor immunotherapy. The clinical treatment of Aurora-A molecular inhibitors is currently in trial [15].

Nuclear protein, coactivator of histone transcription (NPAT) is located in the special structure of histone pedestal in the nucleus and is a crucial molecule in modulating histone transcription and cell cycle [16]. In human ESCs (hESCs), cyclin D2 phosphorylates NPAT, which is a key factor necessary for self-renewal and maintenance of stemness of hESC [17]. A few studies have pointed out that NPAT has a certain correlation with the development of tumors. Compared with normal B cells, NPAT protein expression is significantly down-regulated in B cells of chronic lymphocytic leukemia, which may be related to the increased degradation of NPAT protein or the inhibition of gene transcription, but the specific modulatory mechanism remains to be explored [18]. As reported previously, the anti-cancer drug lignan arctigenin selectively down-regulates the expression of histone synthesis-related proteins such as NPAT, blocks cancer cells in the G0/G1 phase, followed by specifically inducing cancer cells apoptosis through the Akt-1 signaling pathway [19]. This down-regulation process may be achieved by inhibiting the cyclin E/CDK2 or cyclin H/CDK7 signaling pathway, resulting in the failure of NPAT phosphorylation. Further research is needed on the correlation between NPAT and LC. In a range of malignancies, CDKs has demonstrated important regulatory effects on radiation therapy [20, 21]. Therefore, by focusing on and controlling the cell cycle, it would be worthwhile to investigate in the future whether NPAT may be transformed into a novel target for anti-cancer radiation therapy.

Hence, this study probed the regulatory mechanism of Aurora-A influencing the stemness in LC cells through modulating NPAT, so as to provide a new experimental basis and target for finally clarifying the molecular mechanism and clinical treatment of highly aggressive and highly metastatic LC. We also reveal the underlying mechanism of Aurora-A/NPAT/Wnt-β-catenin in LC cell proliferation and metastasis, providing a new entry point for future therapeutic approaches.

MATERIAL AND METHODS

Cell culture

Procell (Wuhan, China) supplied LC cell lines (A549, Calu-6, and SK-LU-1) as well as the human normal lung epithelial cell line (BEAS-2B). A549 cells were hatched in Ham's F-12K medium (Gibco, USA), BEAS-2B cells were grown in DMEM (Gibco, USA), and Calu-6 and SK-LU-1 cells were grown in MEM (Gibco, USA). 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco, USA) were administered to each medium, and the mixture was then heated to 37°C with 5% CO_2.

Cell transfection

Via Ribobio (Guangzhou, China), the small interference RNAs (siRNAs) for Aurora-A, NPAT, and si-NC were acquired. GenePharma (Shanghai, China) provided the pcDNA and pcDNA-NPAT. Lipofectamine 2000 (Invitrogen, USA) was implemented for cell transfection.

RT-qPCR

TRIzol reagent (Invitrogen, USA) was utilized for obtaining total RNA from cells. Using the PrimeScipt RT Master Mix kit (Takara, Japan), individual RNA samples were reverse-transcribed into cDNA. Then, qPCR was performed using the SYBR Premix Ex Taq™ (Takara, Japan). Utilizing the $2^{-\Delta\Delta Ct}$ technique computed relative level. Primers for Aurora-A were (5'-3'): forward TGAATAACACCCAAAAGAGCAAG and reverse -ACTTTCCTTTACCCAGAGGGC; NPAT: forward ACTTTCTCAGATCAGGAGCA and reverse TCTGCAATTCCAGTTCTCG; GAPDH: forward CGGAGTCAACGGATTTGGTCGTAT; and reverse AGCCTTCTCCATGGTGGTGAAGAC.

Cell counting kit-8 (CCK-8) for cell proliferation

Cells were taken to seed into a 96-well plate for cultivation. After treating with 10 μL of CCK-8 reagent for 2 h, and the kit was from Dojindo, Japan. A microtiter plate reader was adopted for measuring the optical density at 450 nm through.

Colony formation for cell proliferation

Cells (600 cells/well) were taken to plant into 6-well plates and taken for cultivation for 10 days. Then, cells received fixation using 4% formaldehyde as well as staining using 0.1% crystal violet (MCE, USA). Colonies were photographed and counted.

Transwell assay for cell invasion

Cells were planted into the top chamber of a place (8 μm pore size; Corning, USA) which included specific medium without FBS (the insert was coated with Matrigel for invasion assay and the insert was not coated with Materigel for migration assay), the lower chamber included the medium containing 10% FBS. The cells that were still on the top membrane had been eliminated, and the migrated or invaded cells received fixation using methanol and staining using 0.1% crystal violet, imaged, as well as counted after 24 h.

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Sphere formation for cell stemness

On Corning ultra-low attachment plates (Corning, NY), cells were seeded in media without serum. After two weeks of cultivation, 20 ng/mL EGF, 20 ng/mL FGF, 4 mg/mL heparin, and 2% B27 (Invitrogen, USA) were added, and then the number of spheres was analyzed.

Western blot

Proteins were extracted via RIPA lysis buffer (Invitrogen, USA). Afterwards, the protein was separated utilizing 1% SDS-PAGE. Following the transfer of protein samples onto PVDF membranes (Millipore, USA) and sealing with 5% defatted milk, primary antibodies were then incubated for a night at 4°C. The primary antibodies as follows: anti-OCT4, anti-Nanog, anti-SOX2, anti-β-catenin, anti-H3, anti-C-Myc, anti-Cyclin-D1, and anti-β-actin (Abcam, USA). After then, secondary antibodies (Abcam, USA) were applied to the membranes at the temperature of the room. Finally, using an ECL substrate (Invitrogen, USA), protein bands were apparent.

TOP/FOP-Flash luciferase reporter assay

TOP/FOP-Flash (Genechem, China) was co-transfected into cells as well as the indicated plasmids. As previously mentioned, the TOP/FOP ratio was assessed after the TOP/FOP-Flash data were adjusted to the Renilla reniformis (Promega, USA) reading [22].

In vivo tumor xenograft mouse models

Four-week-old male BALB/c nude mice were donated by Shanghai Jiesijie Experimental Animal Co. Ltd. (Shanghai, China). All the procedures involving animals were performed according to the institutional standard guidelines (SZ-0002AL). BALB/c nude mice were given subcutaneous injections of the transfected A549 cells. The mice were then randomly separated into si-NC group and si-Aurora-A-1 group, and each group had 6 mice. Tumor volume was examined every 7 d for 28 d. The xenograft was then removed, and tumor weight was measured at 28 d.

In vivo lung metastasis mouse models

For 48 hours, A549 cells were pre-transfected with either si-NC or si-Aurora-A-1. Next, mice were given injection of cells into their tail veins. The mice were killed and the tumor nodules on the surface of the lungs were counted using a dissecting microscope and the lung nodules were processed for the hematoxylin and eosin (HE) staining after 8 weeks.

Immunohistochemistry (IHC)

Tumor tissues were sliced into 4-μm thick paraffin-embedded sections, followed by dewaxing as well as antigen retrieval, and then incubating with primary antibody of anti-Ki-67 (Abcam, USA) overnight. Afterwards, secondary antibodies were added for cultivation. Next, the slides were then treated with DAB Stain Kit (ZSBio, China) and stained with DAPI.

HE staining

The lung tissues of mice were taken for fixation with 10% paraformaldehyde, embedding in paraffin, followed by cutting into 4 μm thickness section. Next, the sections were taken for dewaxing using xylene and rehydration by successive gradient concentrations of ethanol. The lung tissue sections were first stained with hematoxylin (Invitrogen, USA) and then with eosin (Invitrogen, USA), followed by analysis using light microscopy.

Statistical analysis

For each of the three separate experiments, the data were presented as mean ± SD. the information was assessed by GraphPad PRISM 6 using the Student's t-test or one-way ANOVA. Comparisons were regarded as significantly different at the value of $p<0.05$.

RESULTS

Aurora-A promotes LC cell proliferation, migration as well as invasion

With the aim to identify Aurora-A expression in LC, RT-qPCR analysis was implemented and an elevation of Aurora-A expression was uncovered in LC cells by comparing with the normal cell line (figure 1A). Aurora-A expression was most abundant in A549 together with Calu-6 cells, thus these two cells were chosen for experimental usage. We firstly forced the depletion of Aurora-A by transfecting si-Aurora-A-1/2 into two selected LC cells (figure 1B). Functionally, Aurora-A knockdown caused retarded the proliferation of two selected LC cells (figure 1C-1D). Besides, Aurora-A silence inhibited the migrating and invading capacities of two selected LC cells (figure 1E).

Aurora-A promotes LC cell stemness

We further assessed the impact of Aurora-A on the stemness of LC cells. The data from sphere formation assay as well as western blot analysis disclosed that the number of sphere-forming cells as well as Nanog, OCT4 and SOX2 protein levels were overtly reduced after Aurora-A knockdown (figure 2A-2B).

NPAT promotes LC cell proliferation, migration along with invasion

NPAT has been identified as a key factor necessary for self-renewal and maintenance of stemness of hESC [23]. Therefore, we conjectured that NPAT might be involved in the promotion of the stemness of LC cells regulated by Aurora-A. NPAT expression in LC cells was firstly detected. As displayed in figure 3A, NPAT presented higher

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expression in LC cells in comparison with the normal cell line, which was consistent with the expression pattern of Aurora-A in LC cells. Afterwards, we discovered that after Aurora-A depletion, NPAT expression was declined in two selected LC cells, implying a positive relation between Aurora-A and NPAT expression in LC cells (figure 3B). For further determining the role of NPAT in LC cells, NPAT expression was depleted in two selected LC cells by transfecting two specific siRNAs against NPAT, and RT-qPCR analysis validated the effective transfection (figure 3C). It was observed that NPAT silence repressed two selected LC cells proliferation (figure 3D-3E). Moreover, NPAT knockdown hindered two selected LC cells migration along with invasion (figure 3F).

NPAT promotes LC cell stemness

Similarly, we found that NPAT reduction obviously repressed the number of sphere-forming cells as well as the Nanog, OCT4 as well as SOX2 protein levels, as manifested in figure 4A-4B.

Aurora-A activates the Wnt-β-catenin via regulating NPAT in LC cells

Previous reports have proved that Aurora-A stimulates the Wnt-β-catenin to participate in the progression of diseases [24]. Therefore, our study further detected whether Aurora-A activated the Wnt -β-catenin in LC cells. TOP/FOP-Flash luciferase reporter assay displayed that Aurora-A reduction inhibited the activity of Wnt/β-catenin signaling pathway (figure 5A). Besides, western blot analysis revealed that silenced Aurora-A weakened the nuclear expression of β-catenin protein, along with C-Myc and Cyclin-D1 expression in two selected LC cells (figure 5B). Since we had proved a positive correlation of Aurora-A and NPAT expression in LC cells, we supposed that Aurora-A might activate the Wnt-β-catenin signaling pathway via regulating NPAT. We overexpressed NPAT expression in two selected LC cells (figure 5C) and discovered that elevation of NPAT offset the reduced luciferase activity of TOP-Flash caused by Aurora-A down-regulation in two selected LC cells (figure 5D). Likewise, the lessened nuclear expression of β-catenin protein, along with the reduced expression of C-Myc and Cyclin-D1 in Aurora-A-depleted two selected LC cells was rescued by co-transfection of pcDNA-NPAT (figure 5E).

Aurora-A promotes LC cell proliferation, migration, invasion along with stemness via regulating NPAT

To further verify, whether Aurora-A promoted LC cell proliferation, migration, invasion along with stemness via regulating NPAT, rescue assays were implemented. As displayed in figure 6A-6C, the suppressed proliferation, migration as well as invasion in Aurora-A-depleted two selected LC cells was reversed upon co-transfection of pcDNA-NPAT. Moreover, Overexpression of NPAT rescued the inhibited number of sphere-forming cells as well as the down-regulated protein levels of Nanog, OCT4 along with SOX2 in two selected LC cells transfected with si-Aurora-A-1 (figure 6D-6E).

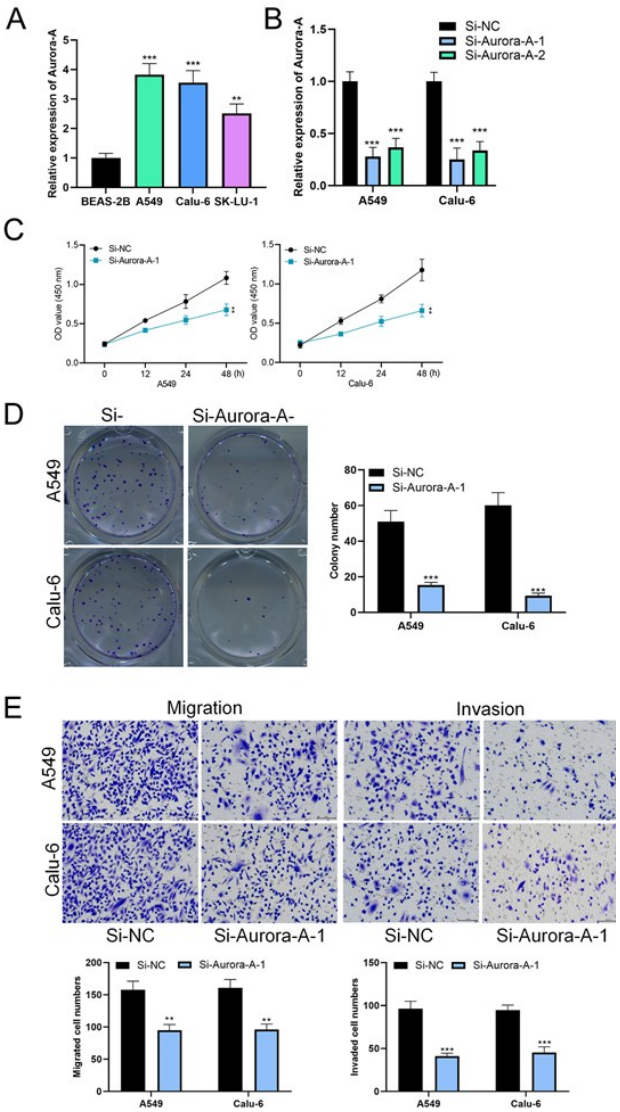


Figure 1. Aurora-A promotes LC cell proliferation, migration along with invasion. **(A);** Aurora-A expression in LC cells along with normal cell line was examined by RT-qPCR. **(B);** Aurora-A expression in two selected LC cells (A549, Calu-6) transfected with siRNAs targeting Aurora-A was detected using RT-qPCR. **(C);** CCK-8 assessed two selected LC cells viability after Aurora-A silence. **(D);** Colony formation assessed two selected LC cells proliferation after Aurora-A silence. **(E);** Transwell assessed two selected LC cells migration and invasion after Aurora-A silence. **P<0.01, ***P<0.001.

Aurora-A promotes tumor growth, metastasis and stemness of LC cells in vivo

The role of Aurora-A in tumor growth of LC was investigated in xenograft models. The mice received injection of stably si-Aurora-A-1-transfected LC cells

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exhibited smaller tumor volume as well as tumor weight (figure 7A-7C). Moreover, IHC presented decreased Ki-67 level in Aurora-A knockdown tumors (figure 7D). To investigate the impact of Aurora-A on cancer metastases *in vivo*, stable si-Aurora-A-1-transfected LC cells were injected into BALB/c nude mice for 8 weeks. The results revealed that Aurora-A silence lessened the numbers of metastatic nodules in lung (figure 7E). Moreover, it was validated that Aurora-A inhibition decreased Nanog, OCT4 along with SOX2 protein levels in tumors of mice (figure 7F).

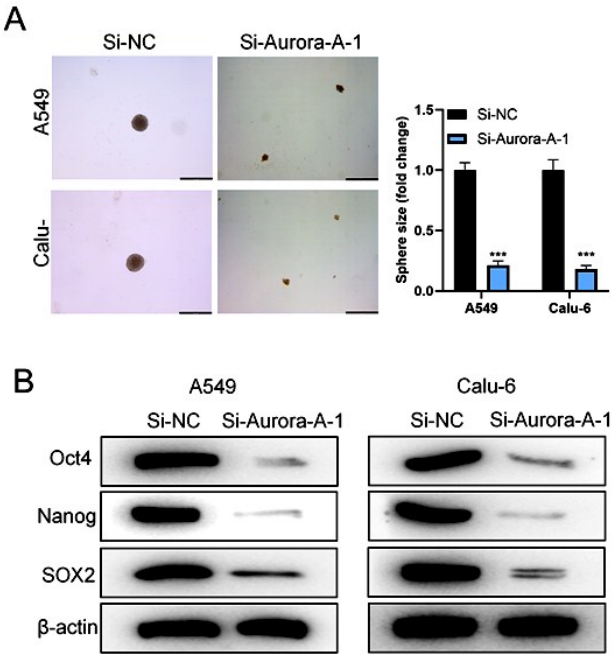


Figure 2. Aurora-A promotes LC cell stemness. **(A);** Sphere formation assessed two selected LC cells (A549, Calu-6) stemness after Aurora-A silence. **(B);** Western blot assessed Oct4, Nanog and SOX2 protein levels in two selected LC cells after Aurora-A silence. ***P<0.001.

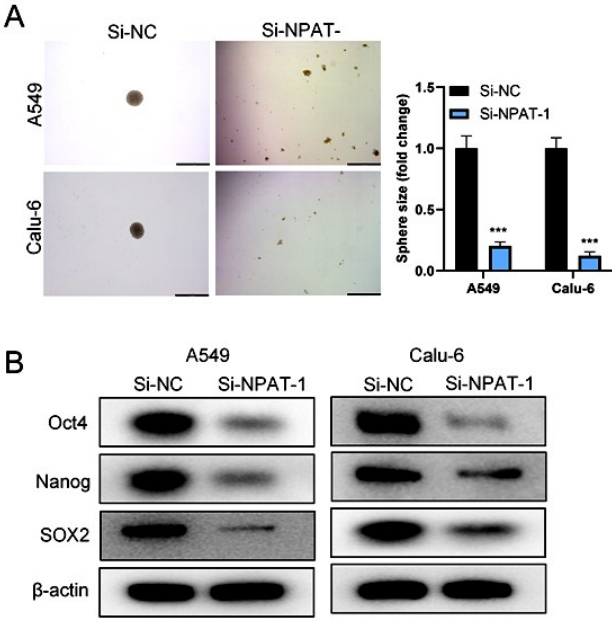


Figure 4. NPAT promotes LC cell stemness. **(A);** Sphere formation assessed two selected LC cells (A549, Calu-6) stemness after NPAT silence. **(B);** Western blot assessed Oct4, Nanog and SOX2 protein levels in two selected LC cells after NPAT silence. ***P<0.001.

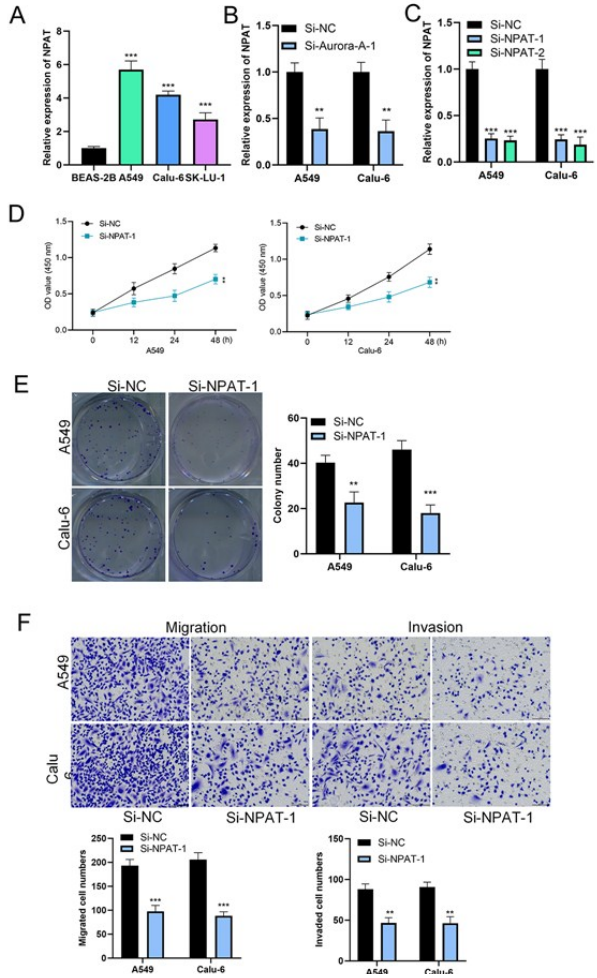


Figure 3. NPAT promotes LC cell malignant behaviors. **(A);** NPAT expression in LC cells along with normal cell line was examined by RT-qPCR. **(B);** NPAT expression in two selected LC cells (A549, Calu-6) transfected with siRNAs targeting Aurora-A was detected by RT-qPCR. **(C);** NPAT expression in two selected LC cells transfected with siRNAs targeting NPAT by RT -qPCR. **(D);** CCK-8 assessed two selected LC cells viability after NPAT silence. **(E);** colony formation assessed two selected LC cells proliferation after NPAT silence. **(F);** Transwell assessed two selected LC cells migration and invasion after NPAT silence. **P<0.01, ***P<0.001.

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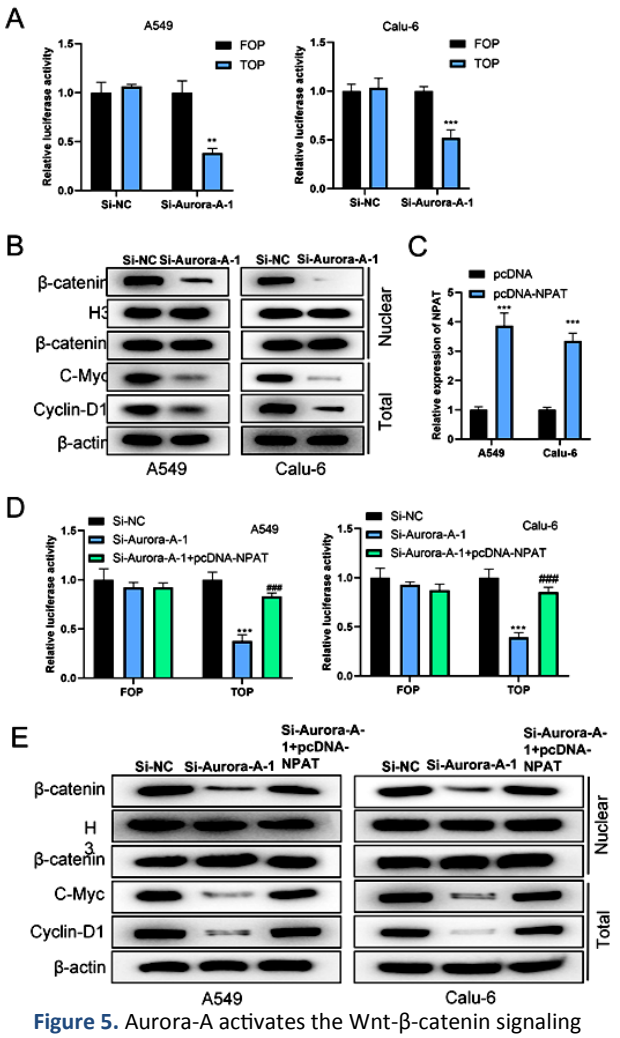


Figure 5. Aurora-A activates the Wnt-β-catenin signaling pathway via regulating NPAT in LC cells. **(A);** TOP/FOP-Flash luciferase reporter assay assessed the luciferase activity of TOP/FOP-Flash in two selected LC cells (A549, Calu-6) after Aurora-A silence. **(B);** Western blot assessed nuclear nuclear expression of β-catenin protein, along with the expression of C -Myc and Cyclin-D1 proteins in two selected LC cells after Aurora-A silence. **(C);** NPAT expression in two selected LC cells transfected with pcDNA-NPAT by RT-qPCR. **(D);** TOP/FOP-Flash luciferase reporter assay assessed the luciferase activity of TOP/FOP-Flash in two selected LC cells after transfection of si-NC, si-Aurora-A-1 or si-Aurora-A-1+pcDNA-NPAT. **(E);** Western blot assessed nuclear nuclear expression of β-catenin protein, along with the expression of C-Myc and Cyclin-D1 proteins in two selected LC cells after transfection of si-NC, si-Aurora-A-1 or si-Aurora-A-1+pcDNA-NPAT. **P<0.01, ***P<0.001, vs Si-NC, ###P<0.001, vs Si-Aurora-A-1+pcDNA-NPAT.

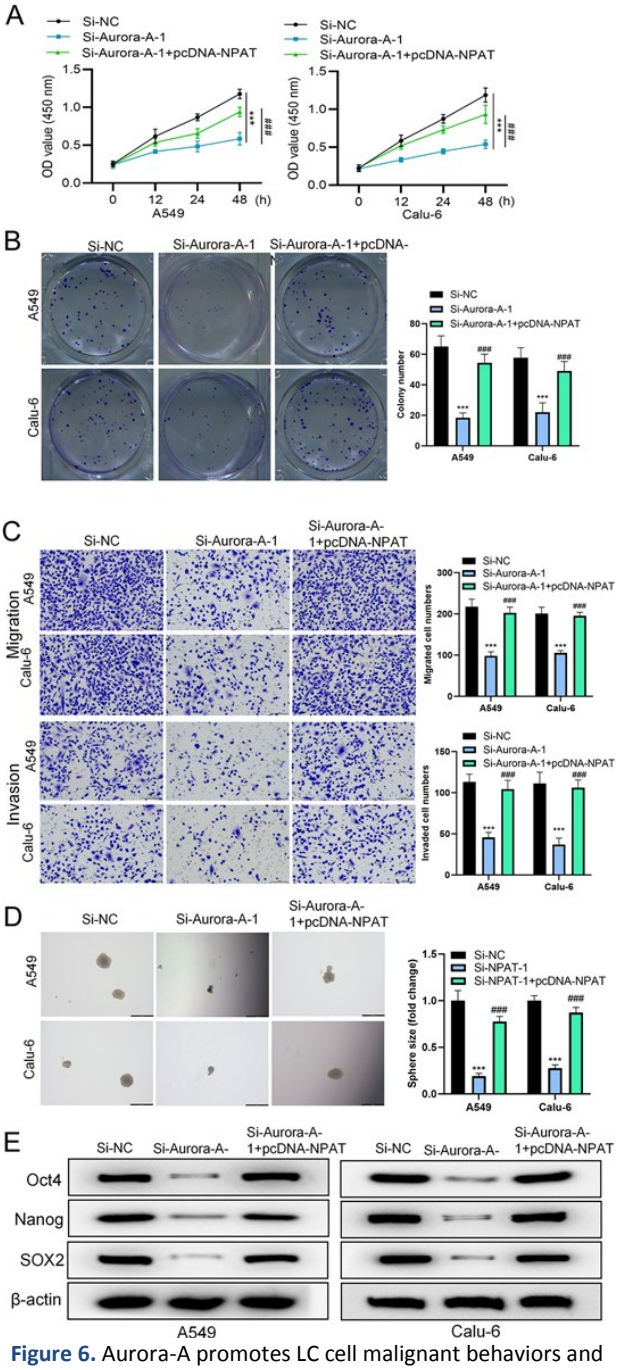


Figure 6. Aurora-A promotes LC cell malignant behaviors and stemness via up-regulating NPAT. **(A-B);** CCK-8 and colony formation assessed two selected LC cells viability after transfection of si-NC, si-Aurora-A-1 and si-Aurora-A-1+pcDNA-NPAT. ©; Transwell assessed two selected LC cells migration and invasion upon transfection of si-NC, si-Aurora-A-1, and si-Aurora-A-1+pcDNA-NPAT. **(D);** Sphere formation assessed two selected LC cells stemness upon transfection of si-NC, si-Aurora-A-1, and si-Aurora-A-1+pcDNA-NPAT. **(E);** Western blot assessed Oct4, Nanog and SOX2 protein levels in two selected LC cells upon transfection of si-NC, si-Aurora-A-1, and si-Aurora-A-1+pcDNA-NPAT. ***P<0.001, vs Si-NC, ###P<0.001, vs Si-Aurora-A-1+pcDNA-NPAT.

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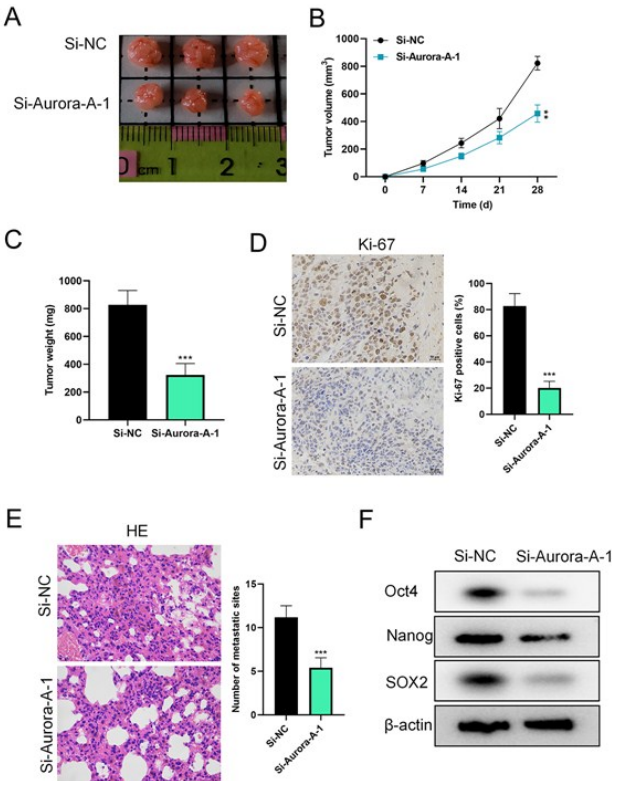


Figure 7. Aurora-A promotes tumor growth, metastasis along with stemness of LC cells in vivo. **(A-C);** si-Aurora-A-1 or si-NC transfected A549 cells were injected into the BALB/c nude mice, followed by measuring tumor volume and weight. **(D);** IHC assessed Ki-67 expression in subcutaneous xenograft tumors after Aurora-A silence. **(E);** HE staining detected number of metastatic nodules in lung after Aurora-A silence. **(F);** Western blot assessed Oct4, Nanog and SOX2 protein levels in tumors after Aurora-A silence. **P<0.01, ***P<0.001.

DISCUSSION

LC patients found at an early stage can receive surgical resection, but most of patients are discovered at the advanced stage and lose the chance for surgery, and can only adopt radiotherapy, chemotherapy, and other therapies [25]. Nevertheless, most patients will have relapse, metastasis, as well as drug resistance after therapy, and the prognosis is very unsatisfactory [26]. Hence, there is an urgent required to explore novel diagnostic markers as well as therapeutic targets for LC. Studies have validated that CSCs also exist in LC and are involved in the development of LC [27]. Nevertheless, the research on targeting LC CSC is still in its infancy, and the regulatory mechanism underlying LC CSC is obscure.

Aurora-A belongs to a key modulator of mitosis and has a crucial role in centrosome function, spindle assembly, as well as mitotic entry [28]. High Aurora-A expression has been certified in a variety of malignancies, LC included [29, 30]. Besides, many reports have correlated Aurora-A expression to poor differentiation, high tumor aggressiveness, as well as lymph node metastasis [31]. Likewise, our study discovered that Aurora-A expression was elevated in LC cells, and silence of Aurora-A apparently hindered LC cell malignant behaviors. Importantly, the elevated Aurora-A expression is linked to high cell proliferation, metastasis and stemness [32], we further explored the impact of Aurora-A inhibition on the stemness of LC cells. Through sphere formation assay and detection of stemness markers, we proved that Aurora-A could promote the stemess of LC, which was in line with previous studies [33]. In addition, in vivo experiments also supported this finding.

NPAT is a main cell cycle regulator that determines the stem cell capacity of hECS [34]. Therefore, our study further investigated the correlation between Aurora-A and NPAT in LC cells. Our study indicated that NPAT was high-expressed in LC cells, and was positively modulated by Aurora-A in LC cells, reflecting that Aurora-A accelerated LC cell stemness via up-regulating NPAT. Further functional assays validated that repression of NPAT hindered LC cell proliferation, migration, invasion, and stemness. All above outcomes suggested the tumor promoting role of NPAT in LC.

The role of Aurora-A as a carcinogenic driver in modulating many molecules as well as signaling pathways has been explored [35]. Accumulating studies have revealed that Aurora-A substrates of GSK3β and β-catenin, also participate in vital carcinogenic signaling [36]. Overexpressed Aurora-A increases GSK3β phosphorylation at Ser9 site, thereby stabilizing β-catenin levels as well as activating Wnt signaling. Stabilized β-catenin is transferred from cell-cell interactions to the nucleus and induces the transcription of target genes [37]. In view of the above supports, our study also looked into the connection between Aurora-A and the Wnt-β-catenin network in light of the aforementioned supports. In line with earlier research, we found that Aurora-A could both activate the Wnt-β-catenin and increase the expression of downstream genes in this pathway [38]. More importantly, our study discovered that Aurora-A activated the Wnt-β-catenin and promoted LC cell proliferation, migration, invasion as well as stemness via up-regulating NPAT.

CONCLUSION

In summary, our study clarifies that Aurora-A/NPAT/Wnt-β-catenin signaling pathway promotes LC cell malignant behaviors along with stemness. Our data suggest that Aurora-A is a key factor in LC progression. The modulation of Aurora-A expression might be an underlying therapeutic approach for LC patients.

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Not applicable.

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Ethical consideration: All the procedures involving animals were performed according to the institutional standard guidelines (SZ-0002AL).

Author contribution: Q.Z. and Y.Z. conceived and designed the experiments. Q.C., P.L. and X.F. contributed significantly to the experiments and arranging data. J.D., Y.T., and Y.M. performed data analyses. Q.C. and Y.Z. wrote the draft manuscript. Q.Z. revised the manuscript. All authors read and approved the final manuscript.

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