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Original Article

Thioridazine promotes primary ciliogenesis in lung cancer cells through enhancing cell autophagy

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Abstract: As a member of the phenothiazine family, thioridazine (THIO) is a powerful anti-anxiety and anti-psychotic drug. In recent years, some research indicated that THIO could suppress the growth of several types of cancer cells. However, the effect of THIO on primary ciliogenesis of cancer cells has not been reported so far. In this study, we mainly investigated the effect of THIO on primary cilia formation of human lung cancer cells. Our results indicated that THIO could promote cell autophagy and further enhance the formation of primary cilia in the cancer cells. During the process, the protein level of BBS4 could be increased in the cancer cells, while the level of OFD1, an inhibitor of BBS4, was reduced obviously. In addition, the cancer cells with more primary cilia also showed weaker invasion ability than normal ones, and further exploration indicated that THIO treatment might inhibit the EMT process in the cancer cells. Therefore, our study indicated that THIO could enhance the primary ciliogenesis of human lung cancer cells through the regulation of cell autophagy and further suppress the invasion ability of cancer cells, providing a promising strategy for the treatment of human lung cancer as well as other cancers in clinical studies.

Keywords: Thioridazine, primary cilium, autophagy, cell invasion

Introduction

Thioridazine (THIO), a member of the phenothiazine family, was originally used to treat psychotic disorders such as psychosis and schizophrenia [1, 2]. In case of advanced cancer, this drug has been used to treat cancer-related sweating [3, 4] as well as depression [5]. In recent years, some studies demonstrated that THIO could inhibit the growth of some cancer cell lines [6-8]. It was also indicated to show selectivity for cancer stem cells [7, 9]. In addition, THIO could induce an increase in p53 expression though neutralizing a crucial regulator of cancer apoptosis, and sensitizes cervical and ovarian cancer cells through the PI3K/Akt/mTOR pathway [6, 10-12]. Thus, THIO is currently regarded as a potential anti-cancer drug administered during chemotherapy [12]. Even though THIO-based therapy is considered promising for cancer chemotherapy, the detail therapy mode and mechanisms still need to be investigated intensively. For example, we are still unclear which kinds of cancers are most sensitive to this drug. Besides, the regulation of its cytotoxic effect on cancer cells and healthy somatic cells also needs our further exploration. Recently, a study indicated that THIO hold an obvious effect to induce autophagy in cancer cells [13], and opened a new sight for the application of THIO in cancer research.

Autophagy is a destructive cellular process to degrade disordered cell organelles as well as some protein aggregates, and it can maintain cellular homeostasis and holds a dual role in cancer development [14-17]. Autophagy was initially regarded as a tumor-suppression mechanism, as autophagy deficiency in mice promoted the tumor initiation. However, some scientists also found that high level of autophagy was required to maintain the high level of metabolism in cancer cells. Therefore, autophagy plays different roles in the process of cell transformation and transformed cell progression [17]. Based on the dual roles of autophagy,

efforts to inhibit or enhance autophagy to improve cancer therapy have thereby attracted great interest in recent years [13, 16, 17].

The primary cilium is a single, microtubule based structure that protrudes from the surface of most mammalian cells, and it functions as a cellular antenna that captures signals from the environment. Interestingly, loss of primary cilium has been observed in kinds of tumors, suggesting a potential suppressive role of the cilium in cancer development [17, 18]. Scientists also have demonstrated that the primary cilium could regulate multiple signaling pathways, such as Shh signaling pathway and Wnt signaling pathway, and the dysfunctions of such functions are associated with a number of cancers [18-21]. More recently, some groups began to unveil the bidirectional interaction between primary cilium and autophagy [22-25]. For example, Tang et al. demonstrated that OFD1 could be degraded by autophagy and further promote cilia formation under the condition of serum starvation. In contrast, the inhibition of autophagy could attenuate satellite OFD1 degradation and lead to lower ciliogenesis rate and shorter cilia, suggesting a positive role of autophagy in ciliogenesis [22]. As the presence of cilia can suppress abnormal cell vitality, the restoration of the primary cilium in cancer cells may be a novel promising approach to attenuate tumor growth [17, 26]. Therefore, the enhancement of autophagy may hold therapeutic action against caner through promoting cilia formation.

Scientists have indicated that THIO hold an obvious effect to induce autophagy in cancer cells and showed therapy effect [13]. However, whether THIO still hold an effect on the formation of primary cilium is unclear. In this study, we mainly explored the role of THIO on the formation of primary cilium in human lung cancer cells.

Material and methods

This study was approved by the Committee on the Ethics of Animal Experiments and Human Subject Research of Southern Medical University, and the Ethics Committees of Southern Medical University have approved this consent procedure.

Cell culture

Human lung cancer cell line (A549) was obtained from the Cell Bank of the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). A549 cells were maintained in F-12K medium (Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco), and penicillin-streptomycin mixed solution. The media were changed every other day. The cells were incubated in a humidified incubator with 5% CO_2 at 37°C, and were passaged by a dilution of 1:4 once every 4 days or 5 days.

Effect of THIO on cell proliferation

The A549 cells were plated in 96-well plates at a concentration of 1.0×10^5 cells/well, and were treated with THIO (Sigma) at different concentrations (0 μM, 10 μM, 20 μM, 50 μM and 100 μM) for 24 h, 48 h, 72 h and 96 h respectively. The medium without any cells was used as the background group. The proliferation index of each group was determined using the CCK-8 method (Dojindo) according to the manufacturer's instructions [27, 28]. The Proliferation index = the absorbance of experimental group - the absorbance of blank group, was used to measure cell proliferation in our study.

Cell invasion assay

For cell invasion assay, a trans-well system (pore size: 8 μm; Euroclone SPA) was used following the manufacturer's procedures. The A549 cells were seeded into the upper insert covered with matrigel at the concentration of 1×10^5 cells per insert in serum-free medium. Outer wells were filled with F-12K medium containing 10% FBS as a chemoattractant. Then, the cells were incubated for 24 hours at 37°C. Non-invading cells were removed by swabbing the top layer, and cells able to migrate through the gel and insert to the lower surface of the membrane were stained with crystal violet. The number of cells in six random fields was counted for each filter.

Immunofluorescence

The cells were fixed in 4% formaldehyde for 20 minutes at 4°C firstly, and further incubated with 0.3% Triton X-100 for 20 minutes at room

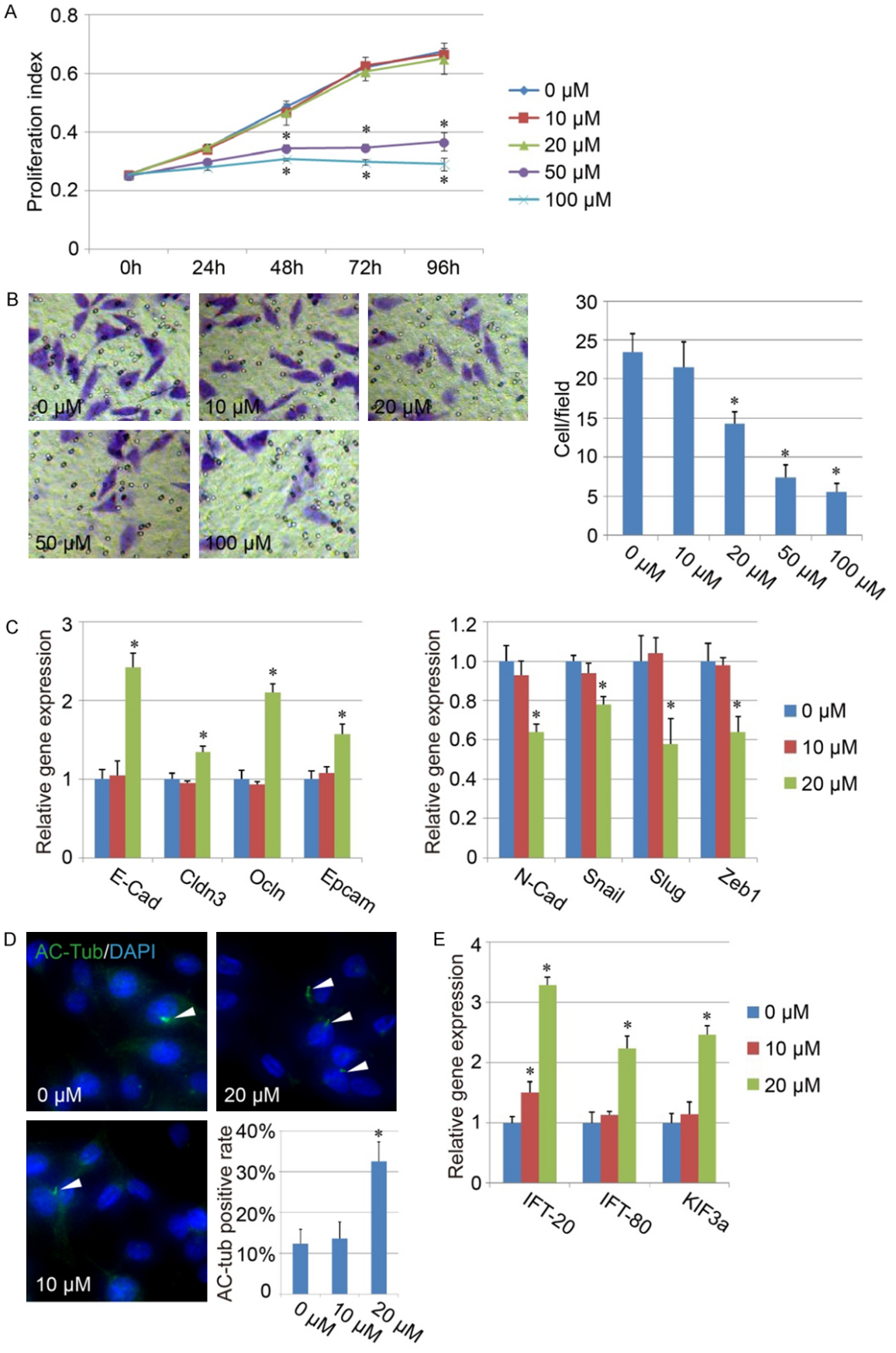
A
Proliferation index
0.8
0.6
0.4
0.2
0
0h
24h
48h
72h
96h
0 μM
10 μM
20 μM
50 μM
100 μM
B
0 μM
10 μM
20 μM
50 μM
100 μM
Cell/field
30
25
20
15
10
5
0
C
Relative gene expression
E-Cad
Cldn3
Ocln
Epcam
N-Cad
Snail
Slug
Zeb1
D
AC-Tub/DAPI
AC-tub positive rate
40%
30%
20%
10%
E
IFT-20
IFT-80
KIF3a

Figure 1. Effect of THIO on cell vitality and primary cilia formation. A. Effect of THIO on the proliferation ability of A549 cells; B. Effect of THIO on the invasion ability of A549 cells; C. qPCR detection of key genes expression during MET; D. Evaluation of primary ciliogenesis via immunofluorescence staining of AC-Tub; E. qPCR detection of key ciliogenesis genes expression. Similar results were obtained in three independent experiments and all of results are expressed as mean ± SEM. For qPCR detection, the gene expression level of the normal cell group was regarded as "1.0", and the relative expression level of the other groups was evaluated. One-way ANOVA followed by a t-test was used to compare the various groups, and $P < 0.05$ was considered statistically significant. *$P < 0.05$ compared with normal A549 cell group.

temperature. To reduce nonspecific background staining, the cells were pre-incubated with PBS containing 5% BSA for 30 minutes at room temperature before staining. For immunofluorescence, the primary antibodies used were Mouse anti-human acetylated tubulin (AC-Tub) monoclonal antibody (1:1000, Sigma). After 12-14 hours of incubation at 4°C, the samples were washed three times with PBS and processed using Goat anti-Mouse IgG Alexa Fluor® 488 secondary antibody (1:2000, Abcam). The mounting medium contained DAPI to highlight the cell nucleus. Finally, the sections were observed with Axio Fluorescence Microscopy (Zeiss, Germany) with AxioCAM MRc5 (Zeiss). The number of positive staining cells in six random fields was counted for each group. The positive staining rate = the number of AC-Tub positive staining cells/the number of DAPI positive staining cells.

Real-time qPCR

For real-time qPCR analysis, 3.0×10^6 cells were homogenized in 1 mL of Trizol reagent (Invitrogen), and total RNA was extracted. For each sample, RNA (2 mg) was reverse-transcribed using an RT-PCR kit (Takara, Japan), and qPCR was performed with a Thermal Cycler DiceTM Real-Time System and SYBR Green Premix EX TaqTM (Takara). In our study, GAPDH was used for qPCR normalization and all items were measured in triplicate. All primer sequences (5'→3') are as follows: E-Cad Forward (F) 5'-CGAGAGCTACACGTTCACGG-3'; Reverse (R) 5'-GGGTGTCGAGGGAAAAATAGG-3'; Cldn3 Forward (F) 5'-AACACCATTATCCGGGACTTCT-3'; Reverse (R) 5'-GCGGAGTAGACGACCTTGG-3'; Ocln Forward (F) 5'-ACAAGCGGTTTTATCCAGAGTC-3'; Reverse (R) 5'-GTCATCCACAGGCGAAGTTAAT-3'; Epcam Forward (F) 5'-AATCGTCAATGCCAGTGTACTT-3'; Reverse (R) 5'-TCTCATCGCAGTCAGGATCATAA-3'; N-Cad Forward (F) 5'-GGCAAGTTGATTGGAGGGATG-3'; Reverse (R) 5'-TAATGTGCAGGTGCCGGTTCAG-3'; Snail Forward (F) 5'-ACTGCAACAAGGAATACCTCAG-3'; Reverse (R) 5'-GCACTGGTACTTCTTGACATCTG-3'; Slug Forward (F) 5'-TGTGACAAGGAATATGTGAGCC-3'; Reverse (R) 5'-TGAGCCCTCAGATTTGACCTG-3'; Zeb1 Forward (F) 5'-TTACACCTTTGCATACAGAACCC-3'; Reverse (R) 5'-TTTACGATTACACCCAGACTGC-3'; IFT20 Forward (F) 5'-GCAGCAACTTCAAGCCCTAAT-3'; Reverse (R) 5'-ACGCCACCTCTTGTGACATAG-3'; IFT88 Forward (F) 5'-GCCGAAGCACTTAACACTTAT-3'; Reverse (R) 5'-GTCTAATGCCATTCGGTAGAA-3'; KIF3a Forward (F) 5'-GAGGAGAGTCTGCGTCAGTCT-3'; Reverse (R) 5'-CAGGCTTTGCAGAACGCTTTC-3'; GAPDH Forward (F) 5'-ACCACAGTCCATGCCATCAC-3'; Reverse (R) 5'-TCCACCACCCTGTTGCTGTA-3'.

Western blot

The cell samples were harvested with RIPA lysis buffer and the protein content of cell lysates in each group was further determined using BCA protein estimation kit (Pierce, USA). Equal amounts (20 mg) of protein were loaded per lane and electrophoresed in a 10% acrylamide gel (120 V for 1 h). The protein transfer was further performed using nitrocellulose for 1 h at 100 V. The primary antibodies used were anti-LC3 (1:500; Santa), anti-p62 (1:500; Santa), anti-BBS4 (1:300; Santa), anti-OFD1 (1:400; Santa), and anti-GAPDH (1:1000; Santa). Anti-mouse, rabbit or goat HRP and an Amersham ECL kit (GE Healthcare) were further used to detect protein.

Statistical analysis

The results are expressed as means ± SEM, and statistical analysis was performed with SPSS 17.0. The differences among groups were analyzed by one-way ANOVA followed by t-tests. $P < 0.05$ was considered statistically significant.

Results

THIO inhibited A549 cell vitality and primary cilia formation

THIO has been demonstrated to hold toxicity to cancer cells. Our result also confirmed such

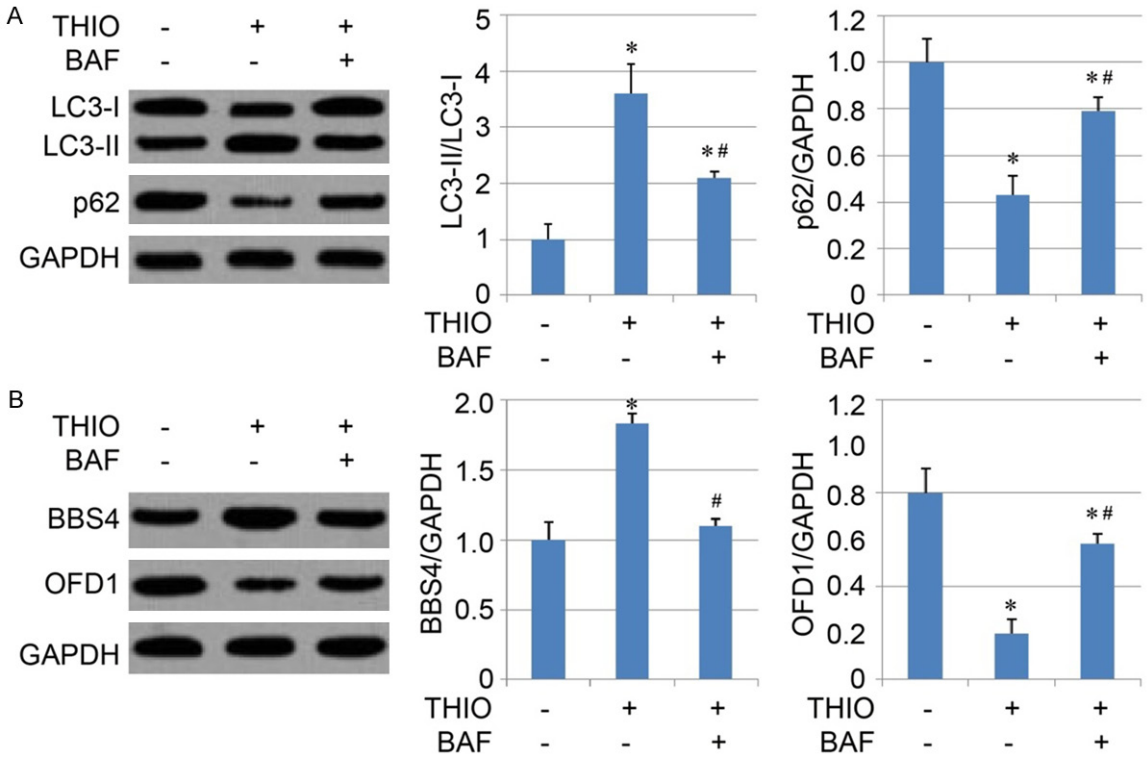

Figure 2. Evaluation of autophagy in cells treated with THIO and BAF. A. Western blot detection of autophagy protein (p62 and LC-3) in cancer cells; B. Analysis of the BBS4-OFD1 pathway in cancer cells. Human A549 cells were treated with THIO (20 μM) and BAF (50 nM) for 24 hours. The relative intensity level of the normal cell group was regarded as "1.0", and the relative level of the other groups was evaluated. Similar results were obtained in three independent experiments and all of results are expressed as mean ± SEM. One-way ANOVA followed by a t-test was used to compare the various groups, and $P < 0.05$ was considered statistically significant. *$P < 0.05$ compared with normal A549 cell group. #$P < 0.05$ compared with THIO group.

conclusion and indicated that the proliferation ability of A549 cells could be inhibited with the treatment of THIO at the concentration of 50 μM and 100 μM compared with normal cells obviously (P < 0.05). However, THIO at the concentration of 10 μM and 20 μM couldn't influence cell proliferation (**Figure 1A**). To avoid the influence of THIO cytotoxicity at high concentration on cell proliferation and cell cycle, low concentration was chosen for our further study. Cell invasion assay indicated that THIO at the concentration of 20 μM could inhibit cell invasion ability, even though the concentration could not affect cell proliferation. However, THIO at the concentration of 10 μM showed no obvious effect on the invasion of A549 cells (**Figure 1B**). As epithelial mesenchymal transition (EMT) was always regarded as the key process for cancer cell invasion, we detected some key genes expression about EMT herein, and found that the expression of epithelial genes (E-cad, Cldn3, Ocln and Epcam) could be up-regulated with the treatment of THIO at the concentration of 20 μM, while the expression of mesenchymal genes (N-cad, Snail, Slug and Zeb1) were reduced obviously (P < 0.05). However, the expression of either epithelial genes or mesenchymal genes was not influenced by THIO at the concentration of 10 μM (**Figure 1C**), suggesting that 20 μM might be the best concentration for our further research. Then, we analyzed the formation of primary ciliausing immunofluorescence and qPCR. AC-Tub was used to label primary cilia on A549 cells, and the staining results indicated that the positive rate of AC-Tub was increased significantly with the treatment of THIO (20 μM) compared with the control group (P < 0.05), even though the positive staining could be found in all groups. Besides, the treatment of THIO also could up-regulated the expression of key ciliogenesis genes, IFT-20, IFT-88 and KIF3a in A549 cells at the concentration of 20 μM. However, the group of THIO at 10 μM didn't hold

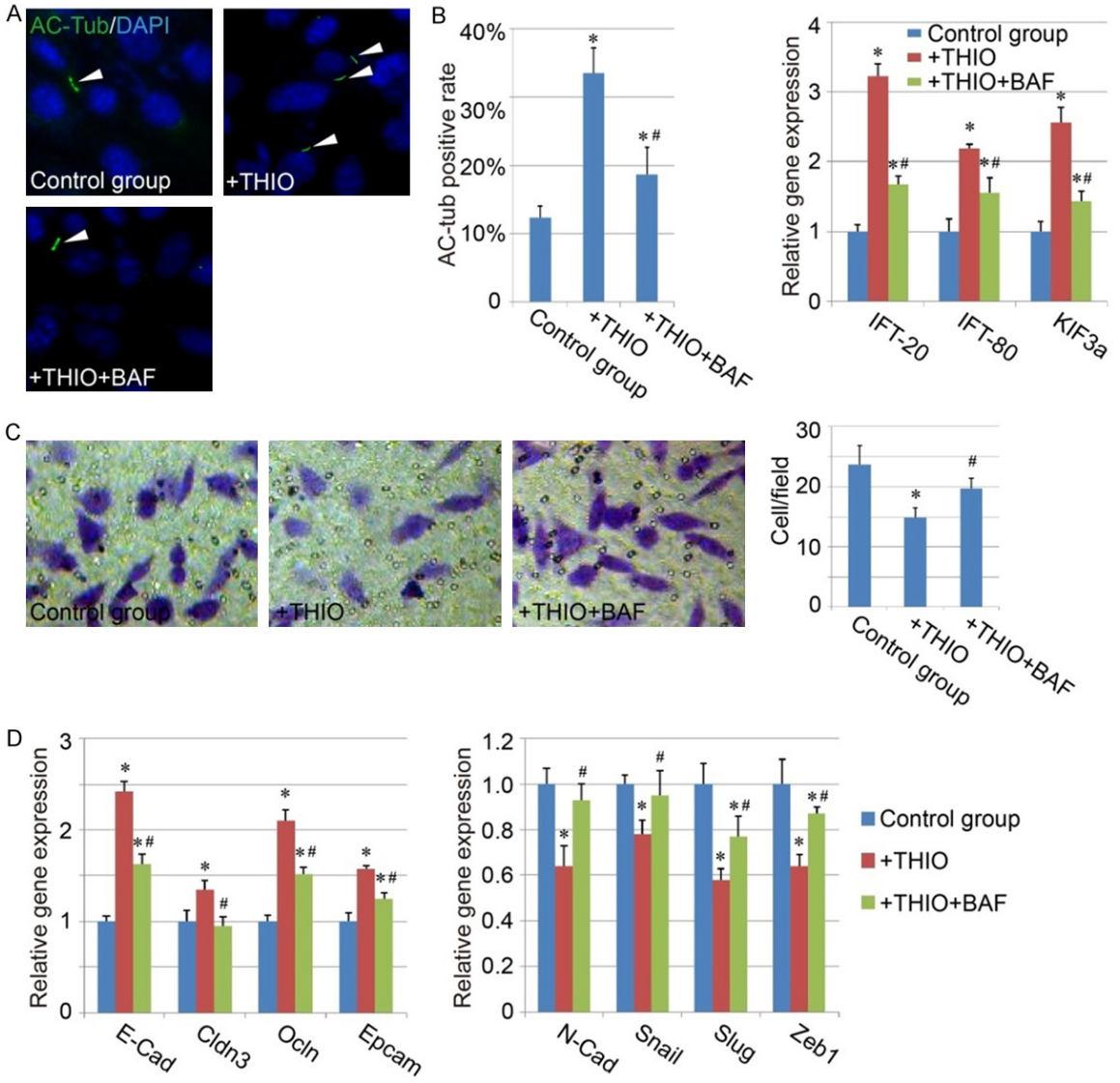

Figure 3. The rescue effect of BAF on primary ciliogenesis and cell invasion. A. Evaluation of primary ciliogenesis via immunofluorescence staining of AC-Tub; B. qPCR detection of key ciliogenesis genes expression; C. Evaluation of the invasion ability of A549 cells treated with ThIO and BAF; D. qPCR detection of key genes expression during MET. For qPCR detection, the gene expression level of the normal cell group was regarded as "1.0", and the relative expression level of the other groups was evaluated. One-way ANOVA followed by a t-test was used to compare the various groups, and $P < 0.05$ was considered statistically significant. *$P < 0.05$ compared with normal A549 cell group. #$P < 0.05$ compared with THIO group.

obvious effect on primary cilia formation (**Figure 1D**, **1E**).

THIO regulated primary ciliogenesis and cell invasion through cell autophagy

We further evaluated cell autophagy in our study, and our results showed that the content of p62 in A549 cells could be reduced with the treatment of THIO, and the level of LC3-II could be increased, accompanied with the decreased LC3-I (**Figure 2A**). Therefore, the ratio of LC3-II/LC3-I in THIO group was much higher than control group (P < 0.05). Thus, similar to the previous research, our study also indicated that THIO hold the function to induce autophagy in human cancer cells. Scientists have demonstrated that OFD1 at centriolar satellites functions as a suppressor of primary ciliogenesis through sequestering BBS4, and autophagy can remove OFD1 from the pericentrosomal

compartment via the interaction with LC3, and further allows BBS4 translocation and cilia induction [22, 25]. To further confirm the function of THIO on primary ciliogenesis in A549 cells, we detected OFD1 and BBS4 in protein level herein. The results indicated that the protein level of BBS4 was increased with the treatment of THIO, while the level of OFD1 was reduced obviously ($P < 0.05$), indicating that THIO could enhance primary ciliogenesis in A549 cells via autophagy and BBS4-OFD1 pathway (**Figure 2B**).

To further confirm that the function of THIO on primary ciliogenesis was mainly through autophagy, we used Bafilomycin A1 (BAF) to inhibit autophagy in cancer cells. Our results showed that BAF could up-regulated the protein level of p62 and inhibit the transformation from LC3-I to LC3-II in THIO treated cells. In addition, the regulation of THIO on BBS4 and OFD1 was also inhibited by BAF obviously ($P < 0.05$) (**Figure 2A**, **2B**). We further detected the primary ciliogenesis ability of A549 cells with IF staining and qPCR, and we found that the positive rate of AC-Tub was decreased significantly with the treatment of BAF and THIO (20 μM) compared with the cells treated with THIO only, even though the positive rate in double treatments group was still higher than control group ($P < 0.05$) (**Figure 3A**). Similar to the staining results, the expression of key ciliogenesis genes, IFT-20, IFT-88 and KIF3a, was also down-regulated in the cells treated with BAF and THIO than that treated with THIO only (**Figure 3B**). We further analyzed the cell invasion ability in different groups, and the results of cell invasion assay indicated the treatment of BAF could rescue the inhibition of THIO on A549 cancer cells in some degree, no significant difference could be found between the double treatment group and the control group (**Figure 3C**). The qPCR result also showed that the expression of epithelial genes (E-cad, Cldn3, Ocln and Epcam) could be down-regulated in the double treatment group, while the expression of mesenchymal genes (N-cad, Snail, Slug and Zeb1) were increased the double treatment group obviously, compared with the THIO group ($P < 0.05$) (**Figure 3D**). Therefore, those result indicated that the function of THIO on primary ciliogenesis and cell invasion could be inhibited in autophagy deficiency cells.

Discussion

In recent years, THIO has been reported to hold the potential to suppress cell proliferation and induce cell apoptosis in various types of cancers [2, 10, 12, 29, 30]. It also showed therapeutic action against lung cancer [9, 31]. However, the detailed mechanisms of THIO against human cancers still need to be investigated intensively. Therefore, the present study mainly revealed that THIO could enhance the formation of primary cilia through promoting autophagy. Scientists have demonstrated that the loss of primary cilia existed during the development of tumor, and the restoration of the primary cilia in cancer cells have been regarded as a novel promising approach to attenuate tumor growth as the inhibiting effect of primary cilium on cancer cell vitality [17, 25]. For example, some studies suggested the disturbed tumor ciliogenesis could promote cell invasion ability [17, 32-34]. Our research also confirmed the conclusion and indicated that THIO treatment could enhance the primary ciliogenesis and inhibit cell invasion. Besides, the inhibition of autophagy with BAF could suppress the primary ciliogenesis and increase cell invasion ability of cancer cells. We also found that EMT could be inhibited in A549 cells treated with THIO, and BAF treatment also could rescue the inhibition obviously. However, whether THIO could affect EMT in lung cancer cells directly and lead to the inhibition of cell invasion is still unclear for us and need further exploration.

Ciliogenesis is linked to cell-cycle progression tightly. The primary cilium starts to develop during early G1 phase, and further matures by mid/late G1. It starts to disassemble during G2 phase, and is absent gradually in M phase [35-37]. Therefore, the delay of cell cycle can enhance the formation of primary cilia in theory. THIO has been demonstrated to hold toxicity for kinds of cancer cells [2, 10, 12, 29, 30]. While inhibiting cell proliferation and inducing cell apoptosis, the treatment of THIO still can lead to the delay of cell cycle, and such effect should enhance the primary ciliogenesis, and disturb our analysis of the relationship between autophagy and primary cilia. Therefore, the THIO at low concentration (20 μM), which could enhance primary ciliogenesis and couldn't influ-

ence cell proliferation, was chose for our most tests in our research.

In recent years, few clinical trials about THIO were carried out on human cancer patients [38], although the anti-cancer effect of THIO has been demonstrated in vivo using kinds of mouse models [29, 39, 40]. In the clinical studies of cancer, THIO is mainly applied in cancer patients for managing depression and psychosis [38], and there has been only one case report of successful treatment with high-dose THIO in a cervical cancer patient so far. Because the complex mechanisms of the THIO anti-cancer effect have not been clarified clearly. Besides understanding the therapy mechanisms, we also should pay more attention to the concentration of THIO used in human, because of its side effects, such as movement disorder, cardiac toxicity, and central nervous system effect. Some scientists have indicated that the concentration of THIO needed to effectively inhibit cancer cell apoptosis may be much higher for cancer patients, compared with the usual dose that was used in clinic for management of psychosis or depression [10, 11, 40]. Thus, the toxicity of high-dose THIO used in the treatment of cancer should be evaluated carefully. In our study, the THIO at low concentration was chose and showed the therapy effect against cell invasion obviously, indicating that low-dose THIO might still hold therapeutic action against cancer through promoting autophagy and primary ciliogenesis. Therefore, our study may develop a new therapeutic strategy for the treatment of cancers.

Conclusion

In conclusion, our research mainly showed that THIO could enhance the formation of primary cilia in human lung cancer cells through promoting cell autophagy and BBS4-OFD1 pathway, and further suppress cell invasion via inhibiting EMT process.

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Disclosure of conflict of interest

None.

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References

[1] Zhelev Z, Ohba H, Bakalova R, Hadjimitova V, Ishikawa M, Shinohara Y and Baba Y. Phenothiazines suppress proliferation and induce apoptosis in cultured leukemic cells without any influence on the viability of normal lymphocytes. Phenothiazines and leukemia. Cancer Chemother Pharmacol 2004; 53: 267-275.

[2] Min KJ, Seo BR, Bae YC, Yoo YH and Kwon TK. Antipsychotic agent thioridazine sensitizes renal carcinoma Caki cells to TRAIL-induced apoptosis through reactive oxygen species-mediated inhibition of Akt signaling and down-regulation of Mcl-1 and c-FLIP(L). Cell Death Dis 2014; 5: e1063.

[3] Cowap J and Hardy J. Thioridazine in the management of cancer-related sweating. J Pain Symptom Manage 1998; 15: 266.

[4] Zhukovsky DS. Fever and sweats in the patient with advanced cancer. Hematol Oncol Clin North Am 2002; 16: 579-588, viii.

[5] Ly KL, Chidgey J, Addington-Hall J and Hotopf M. Depression in palliative care: a systematic review. Part 2. Treatment. Palliat Med 2002; 16: 279-284.

[6] Amson R, Pece S, Lespagnol A, Vyas R, Mazzarol G, Tosoni D, Colaluca I, Viale G, Rodrigues-Ferreira S, Wynendaele J, Chaloin O, Hoebeke J, Marine JC, Di Fiore PP and Telerman A. Reciprocal repression between P53 and TCTP. Nat Med 2012; 18: 91-99.

[7] Sachlos E, Risueno RM, Laronde S, Shapovalova Z, Lee JH, Russell J, Malig M, McNicol JD, Fiebig-Comyn A, Graham M, Levadoux-Martin M, Lee JB, Giacomelli AO, Hassell JA, Fischer-Russell D, Trus MR, Foley R, Leber B, Xenocostas A, Brown ED, Collins TJ and Bhatia M. Identification of drugs including a dopamine receptor antagonist that selectively target cancer stem cells. Cell 2012; 149: 1284-1297.

[8] Ke XY, Ng VW, Gao SJ, Tong YW, Hedrick JL and Yang YY. Co-delivery of thioridazine and doxorubicin using polymeric micelles for targeting both cancer cells and cancer stem cells. Biomaterials 2014; 35: 1096-1108.

[9] Yue H, Huang D, Qin L, Zheng Z, Hua L, Wang G, Huang J and Huang H. Targeting lung cancer stem cells with antipsychological drug thioridazine. Biomed Res Int 2016; 2016: 6709828.
[10] Kang S, Dong SM, Kim BR, Park MS, Trink B, Byun HJ and Rho SB. Thioridazine induces apoptosis by targeting the PI3K/Akt/mTOR pathway in cervical and endometrial cancer cells. Apoptosis 2012; 17: 989-997.
[11] Rho SB, Kim BR and Kang S. A gene signature-based approach identifies thioridazine as an inhibitor of phosphatidylinositol-3'-kinase (PI3K)/AKT pathway in ovarian cancer cells. Gynecol Oncol 2011; 120: 121-127.
[12] Choi AR, Kim JH and Yoon S. Thioridazine specifically sensitizes drug-resistant cancer cells through highly increase in apoptosis and P-gp inhibition. Tumour Biol 2014; 35: 9831-9838.
[13] Cheng HW, Liang YH, Kuo YL, Chuu CP, Lin CY, Lee MH, Wu AT, Yeh CT, Chen EI, Whang-Peng J, Su CL and Huang CY. Identification of thioridazine, an antipsychotic drug, as an antiglioblastoma and anticancer stem cell agent using public gene expression data. Cell Death Dis 2015; 6: e1753.
[14] Takamura A, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguri S, Eishi Y, Hino O, Tanaka K and Mizushima N. Autophagy-deficient mice develop multiple liver tumors. Genes Dev 2011; 25: 795-800.
[15] Karsli-Uzunbas G, Guo JY, Price S, Teng X, Laddha SV, Khor S, Kalaany NY, Jacks T, Chan CS, Rabinowitz JD and White E. Autophagy is required for glucose homeostasis and lung tumor maintenance. Cancer Discov 2014; 4: 914-927.
[16] White E. The role for autophagy in cancer. J Clin Invest 2015; 125: 42-46.
[17] Cao M and Zhong Q. Cilia in autophagy and cancer. Cilia 2015; 5: 4.
[18] Wong SY, Seol AD, So PL, Ermilov AN, Bichakjian CK, Epstein EH Jr, Dlugosz AA and Reiter JF. Primary cilia can both mediate and suppress Hedgehog pathway-dependent tumorigenesis. Nat Med 2009; 15: 1055-1061.
[19] Goetz SC and Anderson KV. The primary cilium: a signalling centre during vertebrate development. Nat Rev Genet 2010; 11: 331-344.
[20] Wallingford JB and Mitchell B. Strange as it may seem: the many links between Wnt signaling, planar cell polarity, and cilia. Genes Dev 2011; 25: 201-213.
[21] Gerdes JM, Liu Y, Zaghloul NA, Leitch CC, Lawson SS, Kato M, Beachy PA, Beales PL, DeMartino GN, Fisher S, Badano JL and Katsanis N. Disruption of the basal body compromises proteasomal function and perturbs intracellular Wnt response. Nat Genet 2007; 39: 1350-1360.
[22] Tang Z, Lin MG, Stowe TR, Chen S, Zhu M, Stearns T, Franco B and Zhong Q. Autophagy promotes primary ciliogenesis by removing OFD1 from centriolar satellites. Nature 2013; 502: 254-257.
[23] Wrighton KH. Cytoskeleton: autophagy and ciliogenesis come together. Nat Rev Mol Cell Biol 2013; 14: 687.
[24] Pampliega O, Orhon I, Patel B, Sridhar S, Diaz-Carretero A, Beau I, Codogno P, Satir BH, Satir P and Cuervo AM. Functional interaction between autophagy and ciliogenesis. Nature 2013; 502: 194-200.
[25] Tang Z, Zhu M and Zhong Q. Self-eating to remove cilia roadblock. Autophagy 2014; 10: 379-381.
[26] Khan NA, Willemarck N, Talebi A, Marchand A, Binda MM, Dehairs J, Rueda-Rincon N, Daniels VW, Bagadi M, Raj DB, Vanderhoydonc F, Munck S, Chaltin P and Swinnen JV. Identification of drugs that restore primary cilium expression in cancer cells. Oncotarget 2016; 7: 9975-9992.
[27] Liu P, Feng Y, Dong C, Yang D, Li B, Chen X, Zhang Z, Wang Y, Zhou Y and Zhao L. Administration of BMSCs with Muscone in rats with Gentamicin-induced AKI improves their therapeutic efficacy. PLoS One 2014; 9: e97123.
[28] Liu P, Feng Y, Dong C, Liu D, Wu X, Wu H, Lv P and Zhou Y. Study on therapeutic action of bone marrow derived mesenchymal stem cell combined with vitamin E against acute kidney injury in rats. Life Sci 2013; 92: 829-837.
[29] Mu J, Xu H, Yang Y, Huang W, Xiao J, Li M, Tan Z, Ding Q, Zhang L, Lu J, Wu X and Liu Y. Thioridazine, an antipsychotic drug, elicits potent antitumor effects in gastric cancer. Oncol Rep 2014; 31: 2107-2114.
[30] Tuynder M, Fiucci G, Prieur S, Lespagnol A, Geant A, Beaucourt S, Duflaut D, Besse S, Susini L, Cavarelli J, Moras D, Amson R and Telerman A. Translationally controlled tumor protein is a target of tumor reversion. Proc Natl Acad Sci U S A 2004; 101: 15364-15369.
[31] Sudo M, Mori S, Madan V, Yang H, Leong G and Koeffler HP. Short-hairpin RNA library: identification of therapeutic partners for gefitinib-resistant non-small cell lung cancer. Oncotarget 2015; 6: 814-824.
[32] Hassounah NB, Nagle R, Saboda K, Roe DJ, Dalkin BL and McDermott KM. Primary cilia are lost in preinvasive and invasive prostate cancer. PLoS One 2013; 8: e68521.
[33] Xiang W, Jiang T, Guo F, Gong C, Yang K, Wu Y, Huang X, Cheng W and Xu K. Hedgehog pathway inhibitor-4 suppresses malignant properties of chondrosarcoma cells by disturbing tumor ciliogenesis. Oncol Rep 2014; 32: 1622-1630.

[34] Xiang W, Jiang T, Guo F, Xu T, Gong C, Cheng P, Zhao L, Cheng W and Xu K. Evaluating the role of PTH in promotion of chondrosarcoma cell proliferation and invasion by inhibiting primary cilia expression. Int J Mol Sci 2014; 15: 19816-19831.

[35] Pan J and Snell W. The primary cilium: keeper of the key to cell division. Cell 2007; 129: 1255-1257.

[36] Plotnikova OV, Pugacheva EN and Golemis EA. Primary cilia and the cell cycle. Methods Cell Biol 2009; 94: 137-160.

[37] Zhou J. Polycystins and primary cilia: primers for cell cycle progression. Annu Rev Physiol 2009; 71: 83-113.

[38] Hercbergs A. Thioridazine: a radiation enhancer in advanced cervical cancer? Lancet 1988; 2: 737.

[39] Basta-Kaim A, Budziszewska B, Jagla G, Nowak W, Kubera M and Lason W. Inhibitory effect of antipsychotic drugs on the Con A- and LPS-induced proliferative activity of mouse splenocytes: a possible mechanism of action. J Physiol Pharmacol 2006; 57: 247-264.

[40] Park MS, Dong SM, Kim BR, Seo SH, Kang S, Lee EJ, Lee SH and Rho SB. Thioridazine inhibits angiogenesis and tumor growth by targeting the VEGFR-2/PI3K/mTOR pathway in ovarian cancer xenografts. Oncotarget 2014; 5: 4929-4934.