European Review for Medical and Pharmacological Sciences 2019; 23: 2880-2887

LncRNA PVT1 knockdown affects proliferation and apoptosis of uveal melanoma cells by inhibiting EZH2

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Abstract. – OBJECTIVE: To detect the expression of long non-coding ribonucleic acid (lncRNA) plasmacytoma variant translocation gene 1 (PVT1) in uveal melanoma (UM) tissues, and to investigate its influence on the proliferation and apoptosis of UM cells as well as its mechanism.

PATIENTS AND METHODS: 40 cases of UM tissues and 40 cases of adjacent tissues surgically resected in our hospital from October 2015 to April 2018 were collected. The expression level of lncRNA PVT1 in these tissues was determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Stable knockdown of lncRNA PVT1 was constructed in human UM cell line OCM-1 using small interfering RNA (siRNA). The impact of lncRNA PVT1 on UM cell proliferation was detected by Cell Counting kit-8 (CCK-8) and colony formation assay. Flow cytometry was applied to measure the apoptotic level of UM cells in the blank control group and lncRNA PVT1 knockdown group. Meanwhile, the expression level of enhancer of zeste homologue 2 (EZH2) was determined by Western blotting.

RESULTS: The expression level of lncRNA PVT1 in UM tissues was remarkably higher than that in the adjacent tissues ($p<0.05$). UM cell proliferation was notably repressed after lncRNA PVT1 knockdown by siRNA. Flow cytometry results indicated that the number of apoptotic UM cells in lncRNA PVT1 knockdown group significantly increased compared with that in the blank control group ($p<0.05$). The protein expression of EZH2 was suppressed after lncRNA PVT1 knockdown ($p<0.05$).

CONCLUSIONS: LncRNA PVT1 knockdown in UM cells can repress the proliferation of UM cells and promote their apoptosis by regulating EZH2 expression.

Key Words:
Uveal melanoma, LncRNA PVT1, Proliferation, Apoptosis, EZH2.

Introduction

Uveal melanoma (UM) is the most common primary intraocular tumor in adults, accounting for about 5% among various melanomas[1]. Although it is not common in the general population, with an incidence rate of merely 5.1/1,000,000, the disease poses great threats to visual acuity and even life[2]. Approximately a half of patients with primary UM experience tumor metastasis, of which the most common route of metastasis is vascular dissemination[3]. Currently, significant progress has been achieved in the diagnosis of primary UM, and the diagnostic accuracy of choroidal melanoma exceeds 99%[4]. Although the local recurrence of primary UM can be effectively prevented by chemotherapy, radiotherapy and enucleation of eyeballs, efficacious therapeutic method for metastatic UM is lacking. Moreover, the 5-year survival rate of UM patients has not been significantly improved over the past 40 years[5].

Enhancer of zeste homologue 2 (EZH2), a member of the polycomb group (PcG), can regulate cell cycle through nucleosome modification, chromatin remodeling and interaction with other transcription factors. Its expression will be delayed once the tissues maturate or differentiate[6,7]. Studies have manifested that the overexpression of EZH2 is closely related to the proliferation and poor prognosis of many tumor cells, such as breast cancer, endometrial cancer, prostate cancer and UM[8-10]. Therefore, EZH2 may be a potential target for treatment of UM. Long non-coding ribonucleic acids (lncRNAs) are RNA molecules with a transcript length of over 200 nucleotides[11]. LncRNAs themselves cannot encode corresponding proteins in cells, but they can regulate expressions of the corresponding target genes at post-tran-

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scriptional level, epigenetic level and others, ultimately affecting the occurrence and development of diseases[12]. The roles of lncRNA plasmacytoma variant translocation gene 1 (PVT1), a member of lncRNA family, in the occurrence and development of UM have not been reported yet.

In this research, the expression level of lncRNA PVT1 in the clinical specimens of UM patients was detected first. Potential regulatory effects of lncRNA PVT1 on UM cell proliferation and apoptosis were discussed by designing *in vitro* experiments in UM cell line OCM-1.

Patients and Methods

Tissue Specimens

40 cases of UM specimens and 40 cases of adjacent tissue specimens surgically resected in our hospital from October 2015 to April 2018 were collected. After washing with normal saline, all the specimens were cut into pieces and put into Eppendorf (EP) tubes, which were then stored in a refrigerator at -80°C. All these procedures were approved by the Medical Ethics Committee of the Tianjin Medical University Eye Hospital.

Materials

Human UM cell line OCM-1 was purchased from the Institute of Microbiology, Chinese Academy of Sciences (Shanghai, China). Phosphate-Buffered Saline (PBS), trypsin, fetal bovine serum (FBS) and Roswell Park Memorial Institute-1640 (RPMI-1640) medium were bought from Gibco (Grand Island, NY, USA); small interfering RNA (siRNA) from Guge Bio-Technology Co., Ltd. (Wuhan, China). OCM-1 cells were cultured in a cell incubator with 5% CO_2 at 37°C, and subjected to digestion and subculture with 0.25% trypsin-ethylenediamine tetraacetic acid (EDTA) (Thermo Fisher Scientific, Waltham, MA, USA).

PVT1 Knockdown

OCM-1 cells in logarithmic growth phase were immediately digested and inoculated in a 6-well plate. 12 h later, cells with the confluence of 60-80% were washed with serum-free medium 2-3 times and subjected to serum starvation for synchronous growth. Next, PVT1 siRNA was dissolved in RNase-free deionized water at a final concentration of 20 μmol/L. Cells were divided into 2 groups, namely, control group [negative control (NC)-siRNA group] and OCM-1 knockdown group (OCM-1 siRNA group). The prepared transfection solution was added into each well, mixed sufficiently and cultured for another 6 h. The complete medium was replaced. The base sequences of OCM-1 siRNA were as follows: Forward: 5'-GAACGTGAAAGGGAATT-3', Reverse: 5'-AACGTGAATTGACACCCTTT-3'.

Detection of Expression of Relevant Genes Via Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

(1) Total RNA in the tissues or cells was extracted using TRIzol reagent method (Invitrogen, Carlsbad, CA, USA), whose concentration and purity were measured using an ultraviolet spectrophotometer. RNA sample with A260/A280=1.8-2.0 was eligible for use. (2) The mRNA was synthesized into complementary deoxyribonucleic acid (cDNA) through reverse transcription, and the cDNA was stored in the refrigerator at -80°C. (3) RT-PCR system: 2.5 μL 10× Buffer, 2 μL cDNA, 0.25 μL forward primer (20 μmol/L), 0.25 μL reverse primer (20 μmol/L), 0.5 μL dNTPs (10 mmol/L), 0.5 μL Taq polymerase (2×10^6 U/L) and 19 μL ddH_2O.

Western Blotting Assay

(1) Cells were washed with PBS 3 times. (2) 1,000 μL lysis buffer was added into each dish and shaken sufficiently for 20 min. (3) Cells on the bottom of the dish were completely scrapped using a brush and collected into the prepared Eppendorf (EP; Eppendorf, Hamburg, Germany) tubes. (4) The harvested cells were lysed with an ultrasonication instrument for about 15 s. (5) After standing for 15 min, cells were centrifuged at 12,000 r/min for 0.5 h. (6) The supernatant was taken and subpackaged into the EP tubes. The protein concentration was measured by bicinchoninic acid (BCA) method and ultraviolet spectrophotometric assay, and the volume of all sample proteins was maintained at an equal concentration. (7) Proteins were subpackaged and preserved in the refrigerator at -80°C. Total protein of UM cells was extracted and subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After that, the protein was transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Membranes were incubated with primary antibody at 4°C overnight, and goat-anti-rabbit secondary antibody in the dark for 1 h at the other day. Band exposure was developed using an Odyssey membrane scanner. The protein

level was normalized to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Colony Formation Assay

Cells in each group were cultured into the logarithmic growth phase and digested into single-cell suspension with 0.25% trypsin, ensuring a proportion of single cells >95%. Next, the cell suspension was inoculated into the 6-well plate, with about 500 cells in each well. Subsequently, each well was added with 2 mL RPMI-1640 medium, and the liquid was replaced every 48 h. 10 d later, cells were fixed in paraformaldehyde and stained with crystal violet, and the number of colonies in each well was calculated.

Detection of Cell Apoptosis Via Flow Cytometry

UM cells in the logarithmic growth phase were digested, prepared into suspension with 0.25% trypsin-EDTA, and seeded into a 6-well plate. Loading was performed according to the operation steps in the cell apoptosis detection kit Annexin V-FITC (fluorescein isothiocyanate) Propidium Iodide (PI) (Beyotime, Shanghai, China), and the apoptotic rate was calculated.

Cell Counting Kit-8 (CCK-8) Proliferation Assay

Cells in the logarithmic growth phase were inoculated into a 96-well plate and cultured in the incubator with 5% CO_2 at 37°C for 0, 24, 48 and 72 h, respectively. Subsequently, cells were incubated with RPMI-1640 containing 10% Cell Counting Kit-8 (CCK-8) reagent (Dojindo, Kumamoto, Japan) in the dark. After that, 110 μL developing reagent was added into each well of the 96-well plate and incubated at 37°C for 2 h. The absorbance at 450 nm in each group was detected using the ultraviolet spectrophotometer.

5-Ethynyl-2′-Deoxyuridine (EdU) Staining

At 24 h after PVT1 knockdown in OCM-1 cells, Click-iT EdU staining kit (Invitrogen, Carlsbad, CA, USA) was applied to stain the OCM-1 cells according to the procedures. After that, cells in 3 randomly selected fields in each sample were photographed using a fluorescence microscope for counting EdU-positive cells.

Statistical Analysis

Data were analyzed using Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA), and expressed as mean ± standard deviation. The *t*-test was performed for comparison of data between the two groups. $p<0.05$ suggested that the difference was statistically significant.

Results

LncRNA PVT1 Expression in Carcinoma and Adjacent Tissues of UM Patients

First of all, the expression level of lncRNA PVT1 in carcinoma and adjacent tissues of UM patients was measured. As shown in Figure 1, RT-PCR results showed that the expression level of lncRNA PVT1 in UM tissues was remarkably higher (about 9.96 times) than that in the adjacent tissues ($p<0.05$).

Immunohistochemical Staining Results for EZH2 in Carcinoma and Adjacent Tissues of UM Patients

EZH2 expression in carcinoma and adjacent tissues of UM patients was detected *via* immunohistochemical staining. It was indicated that the expression level of EZH2 in UM carcinoma tissues was markedly elevated compared with that in adjacent tissues, suggesting that EZH2 may be a pro-oncogenic protein in UM (Figure 2).

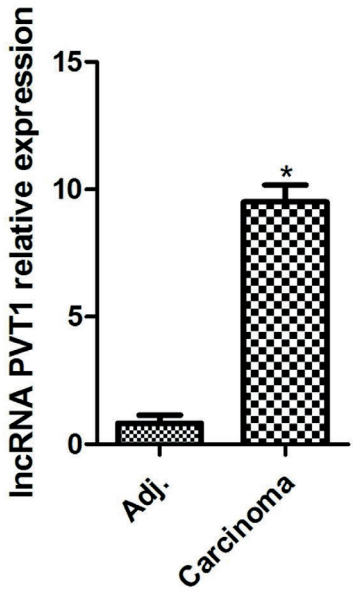

Figure 1. LncRNA PVT1 expression in carcinoma and adjacent tissues of UM patients. Adj.: adjacent tissue, Carcinoma: carcinoma tissue, *$p<0.05$ *vs.* Adj., with a statistically significant difference.

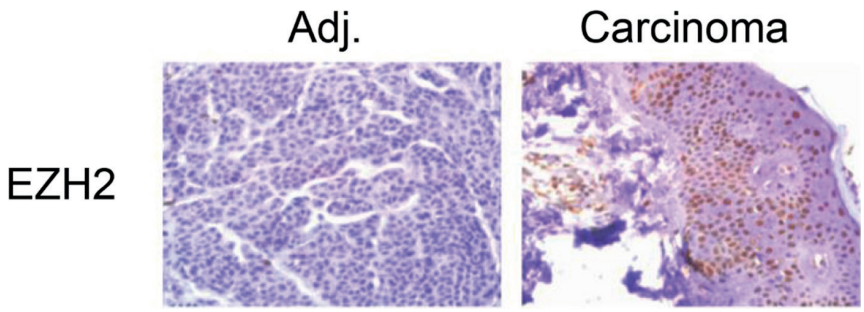

Figure 2. Immunohistochemistry for EZH2 in carcinoma and adjacent tissues of UM patients (Magnification × 100). Adj.: adjacent tissue, Carcinoma: carcinoma tissue.

Identification of UM Cell Lines with LncRNA PVT1 Knockdown

LncRNA PVT1 in UM cell line OCM-1 was knocked down by si-RNA transfection. The results manifested that OCM-1 cells transfected with si-PVT1 had a markedly higher expression level of lncRNA PVT1 than those transfected with si-negative control (NC) ($p<0.05$, Figure 3), indicating that UM cells with lncRNA PVT1 knockdown were successfully constructed.

CCK-8 Proliferation Assay

According to the results of CCK-8 assay, the UM cell proliferation was markedly repressed in the si-PTV1 group at 24, 48 and 72 h, revealing that lncRNA PVT1 knockdown can inhibit the proliferation of UM cells ($p<0.05$, Figure 4).

EdU Staining Results for UM Cells in Each Group

Moreover, UM cell proliferation in each group was assessed by EdU staining. It was shown that the proportion of EdU-positive cells in the si-PVT1 group was evidently lower than that in the si-NC group ($p<0.05$, Figure 5).

Colony Formation Assay Results for UM Cells in Each Group

As shown in Figure 6, the effects of lncRNA PVT1 knockdown on the clonogenic capacity of UM cells were examined through colony formation assay. It was indicated that the clonogenic capacity of UM cells remarkably declined after lncRNA PVT1 was silenced. The number of colonies formed in the si-NC group and si-PTV1 group was (265.56±6.82) *vs.* (44±5.82), respectively ($p<0.05$).

Apoptotic Cells Detected Via Flow Cytometry

The number of apoptotic cells in each group was further determined using flow cytometry. The results indicated that the apoptotic rate of UM cells in the si-NC group and si-PVT1 group were (18.12±1.41)% *vs.* (53.18±2.21)%, respectively ($p<0.05$, Figure 7), revealing that the knockdown of lncRNA PVT1 gene accelerated the apoptosis of UM cells.

Effects of LncRNA PVT Knockdown on EZH2 Protein Expression

Furthermore, the mechanism of lncRNA PVT1 knockdown in influencing UM cell proliferation and apoptosis was investigated. Western blotting was applied to measure the expression level of EZH2 protein. It was manifested that lncRNA PVT1 knockdown was able to downregulate the

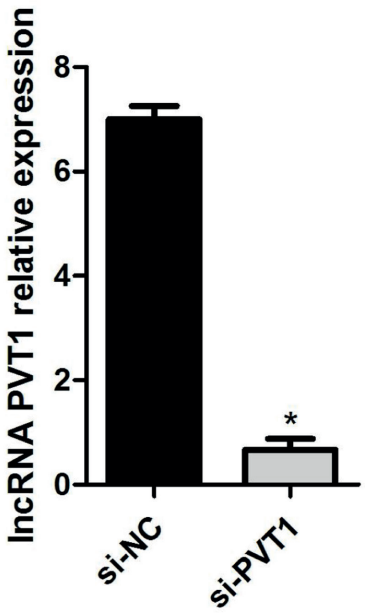

Figure 3. Identification of UM cell lines with lncRNA PVT1 knockdown. si-NC: blank control group, si-PTV1: PVT1 knockdown group, *$p<0.05$ *vs.* si-NC group, with a statistically significant difference.

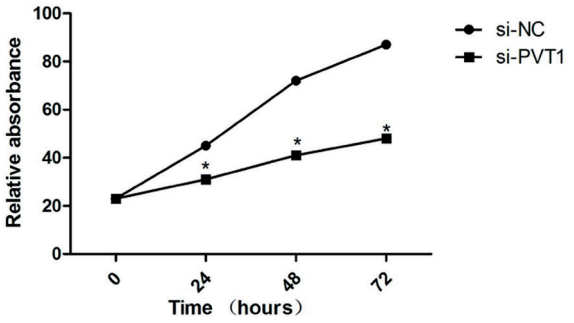

Figure 4. CCK-8 proliferation assay. si-NC: blank control group, si-PTV1: PVT1 knockdown group, *p<0.05 *vs.* si-NC group, with a statistically significant difference.

expression of EZH2 prominently (p<0.05) (Figure 8).

Discussion

As the most common primary intraocular tumor in adults, UM is induced by uveal melanocytes and possesses strong metastatic propensity, with liver, lug and soft tissue as the most common metastatic sites[13,14]. Although the optimal therapeutic methods (surgery or radiotherapy) for primary tumors have been developed, the efficacies on

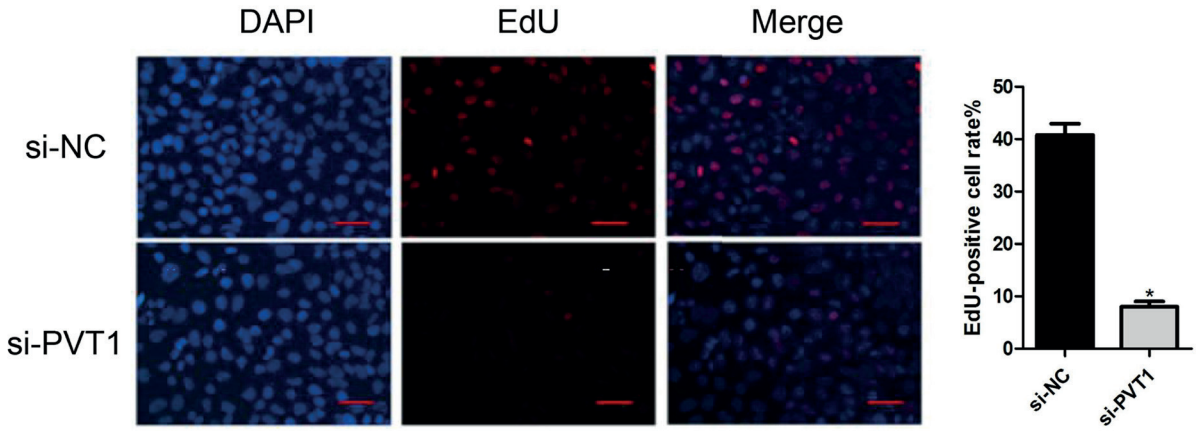

Figure 5. EdU staining results for UM cells in each group. si-NC: blank control group, si-PTV1: PVT1 knockdown group, *p<0.05 *vs.* si-NC group, with a statistically significant difference.

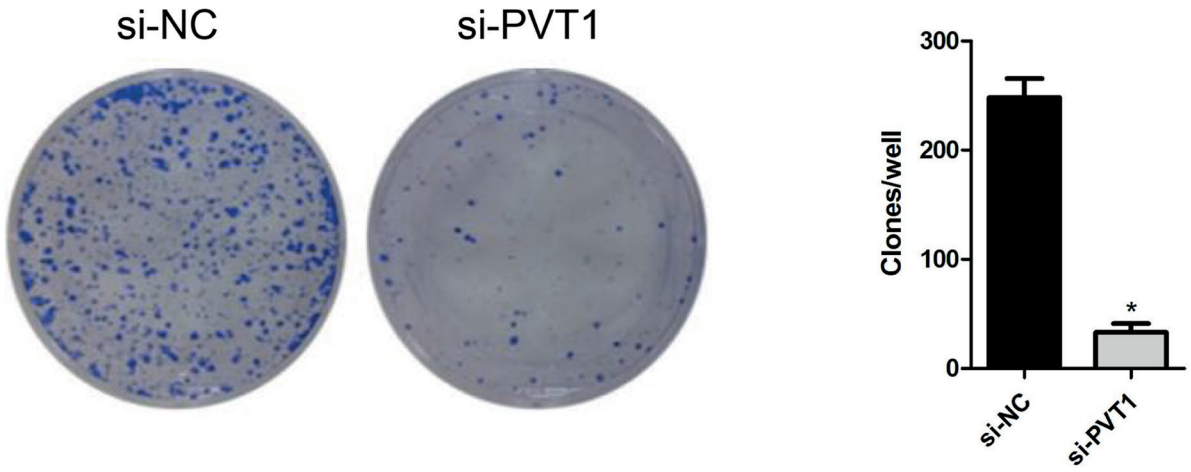

Figure 6. Colony formation assay results for UM cells in each group. si-NC: blank control group, si-PTV1: PVT1 knockdown group, *p<0.05 vs. si-NC group, with a statistically significant difference.

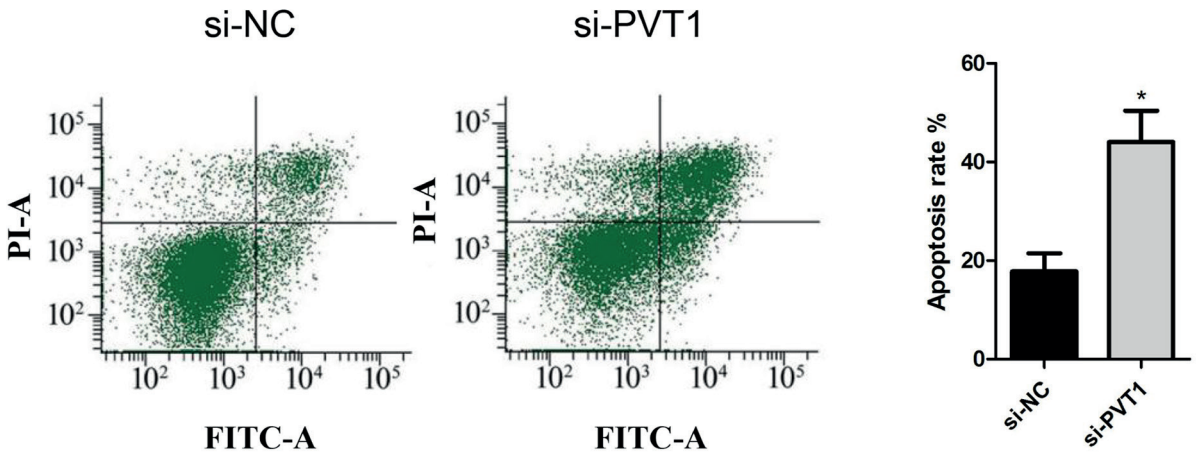

Figure 7. Apoptotic cells detected *via* flow cytometry. si-NC: blank control group, si-PTV1: PVT1 knockdown group, **p*<0.05 *vs.* si-NC group, with a statistically significant difference.

metastatic UM is not satisfactory[15]. A multicenter study on ocular UM reported that the prognosis of metastatic UM is poor, with one-year overall mortality up to 80-87%[16]. The highly metastatic UM is usually triggered by deletion of copy and extra 8q repetition on chromosome 3[17]. Therefore, clarifying the mechanism of the occurrence and development of UM is of great importance for the early prevention and precise treatment.

PcG protein exerts crucial effects on the growth and differentiation of mammalian cells by regulating the expressions of downstream genes. It contains two core complexes, namely, polycomb repressive complex 1 (PRC1) and PRC2[18], of which the former can monoubiquitylate histone H2A at locus Lys 119 through ubiquitin ligases [really interesting new gene 1A (RING1A) and RING1B]. PRC2 can catalyze the monomethylation, dimethylation and trimethylation of histone H3 at locus Lys 27. EZH2, as a histone methyltransferase, can serve as a catalytic subunit of PCR2[19]. It is capable of catalyzing the trimethylation of locus Lys 27 on histone H3 by C-terminal SET domain, further regulating the genes associated with cell proliferation, differentiation and apoptosis, ultimately affecting tumor progression[19]. EZH2 is also a gene with evolutionary conservation, which is discovered in many species and contains similar structural motif and domain in different species (including *Drosophila*, frog, mouse and human)[20]. The mutation and high expression of EZH2 have been observed in multiple malignant tumors, which are closely correlated with the poor prognosis of tumor patients[21,22]. A growing evidence demonstrated that EZH2 expression is controlled by lncRNA. LncRNA LINC00628 can interact with EZH2 in the nucleus and regulate the expression

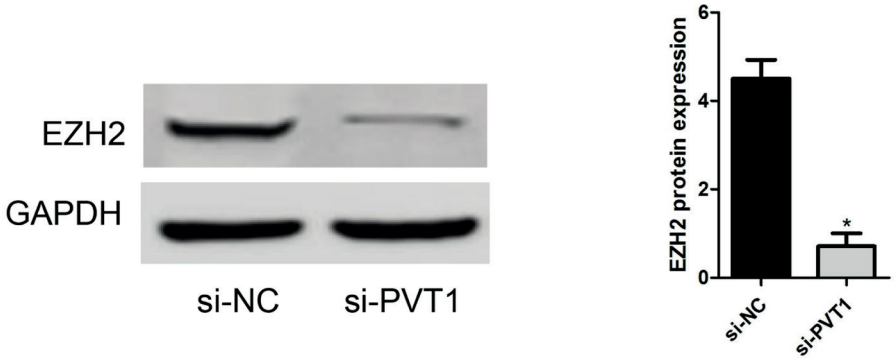

Figure 8. Effects of lncRNA PVT knockdown on EZH2 protein expression. si-NC: blank control group, si-PTV1: PVT1 knockdown group, **p*<0.05 *vs.* si-NC group, with a statistically significant difference.

levels of cell cycle-related genes, thereby inhibiting the proliferation and colony formation of gastric cancer cells and *in vivo* tumorigenesis in mice[23]. In spite of the carcinogenic effect of EZH2 on various types of cancers, several studies have manifested that inhibiting EZH2 can accelerate the progression of some tumors, suggesting that EZH2 also possesses anti-cancer effects. For example, the inactivation of Ser 21 phosphorylation in EZH2 upregulates anti-apoptotic genes (IGF-1, BCL2 and HIF-1α), thus enhancing the cell adhesion-mediated drug resistance in multiple myeloma cells[24]. Loss of EZH2 function can restrain the pancreatic regeneration and promote the Kras-mediated tumor metastasis, indicating that EZH2 can repress tumor progression by homeostatic control of pancreatic regeneration[25]. In this research, the expression level of lncRNA PVT1 in carcinoma and adjacent tissues of UM patients was detected first. The results showed that expression level of lncRNA PVT1 was elevated notably in carcinoma tissues. At the same time, immunohistochemistry results revealed that the expression of EZH2 in carcinoma tissues of UM patients markedly increased. Moreover, proliferation level and clonogenic capacity of UM cells significantly declined after lncRNA PVT1 knockdown. Meanwhile, lncRNA PVT1 knockdown can promote the apoptosis of UM cells remarkably. In addition, we found that EZH2 expression was positively regulated by lncRNA PVT1. Nevertheless, there are certain deficiencies in this research: 1) no animal experiments were designed for verification, and 2) direct targets of lncRNA PVT1 were not found.

Conclusions

We reported for the first time that lncRNA PVT1 knockdown can repress the proliferation and induce the apoptosis of UM cells by inhibiting EZH2 expression.

Conflict of Interest

The Authors declare that they have no conflict of interest.

Funding Acknowledgments

This work was supported by a grant from the National Natural Science Foundation of China (NSFC: 81570872) and Tianjin Medical University Eye Hospital Foundation (16YKYJS0001).

References

1) Field MG, Durante MA, Anbunathan H, Cai LZ, Decatur CL, Bowcock AM, Kurtenbach S, Harbour JW. Punctuated evolution of canonical genomic aberrations in uveal melanoma. Nat Commun 2018; 9: 116.
2) Moore AR, Ran L, Guan Y, Sher JJ, Hitchman TD, Zhang JQ, Hwang C, Walzak EG, Shoushtari AN, Monette S, Murali R, Wiesner T, Griewank KG, Chi P, Chen Y. GNA11 Q209L mouse model reveals RasGRP3 as an essential signaling node in uveal melanoma. Cell Rep 2018; 22: 2455-2468.
3) Yang J, Manson DK, Marr BP, Carvajal RD. Treatment of uveal melanoma: where are we now? Ther Adv Med Oncol 2018; 10: 1961372215.
4) Gong C, Shen J, Fang Z, Qiao L, Feng R, Lin X, Li S. Abnormally expressed JunB transactivated by IL-6/STAT3 signaling promotes uveal melanoma aggressiveness via epithelial-mesenchymal transition. Biosci Rep 2018; 38:
5) Olah G, Dobos N, Vamosi G, Szabo Z, Sipos E, Fodor K, Harda K, Schally AV, Halmos G. Experimental therapy of doxorubicin resistant human uveal melanoma with targeted cytotoxic luteinizing hormone-releasing hormone analog (AN-152). Eur J Pharm Sci 2018; 123: 371-376.
6) Yan KS, Lin CY, Liao TW, Peng CM, Lee SC, Liu YJ, Chan WP, Chou RH. EZH2 in cancer progression and potential application in cancer therapy: a friend or foe? Int J Mol Sci 2017; 18: E1172.
7) Zhou CG, Hui LM, Luo JM. Epigallocatechin gallate inhibits the proliferation and induces apoptosis of multiple myeloma cells via inactivating EZH2. Eur Rev Med Pharmacol Sci 2018; 22: 2093-2098.
8) Beca F, Kensler K, Glass B, Schnitt SJ, Tamimi RM, Beck AH. EZH2 protein expression in normal breast epithelium and risk of breast cancer: results from the nurses' health studies. Breast Cancer Res 2017; 19: 21.
9) Qian K, Liu G, Tang Z, Hu Y, Fang Y, Chen Z, Xu X. The long non-coding RNA NEAT1 interacted with miR-101 modulates breast cancer growth by targeting EZH2. Arch Biochem Biophys 2017; 615: 1-9.
10) Wang ZQ, Cai Q, Hu L, He CY, Li JF, Quan ZW, Liu BY, Li C, Zhu ZG. Long noncoding RNA UCA1 induced by SP1 promotes cell proliferation via recruiting EZH2 and activating AKT pathway in gastric cancer. Cell Death Dis 2017; 8: e2839.
11) Zhan M, He K, Xiao J, Liu F, Wang H, Xia Z, Duan X, Huang R, Li Y, He X, Yin H, Xiang G, Lu L. LncRNA HOXA11-AS promotes hepatocellular carcinoma progression by repressing miR-214-3p. J Cell Mol Med 2018: 10.1111/jcmm.13633.
12) Liang H, Pan Z, Zhao X, Liu L, Sun J, Su X, Xu C, Zhou Y, Zhao D, Xu B, Li X, Yang B, Lu Y, Shan H. LncRNA PFL contributes to cardiac fibrosis by acting as a competing endogenous RNA of let-7d. Theranostics 2018; 8: 1180-1194.
13) Kashyap S, Meel R, Singh L, Singh M. Uveal melanoma. Semin Diagn Pathol 2016; 33: 141-147.

14) Tarlan B, Kiratli H. Uveal melanoma: current trends in diagnosis and management. Turk J Ophthalmol 2016; 46: 123-137.

15) Chattopadhyay C, Kim DW, Gombos DS, Oba J, Qin Y, Williams MD, Esmaeli B, Grimm EA, Wargo JA, Woodman SE, Patel SP. Uveal melanoma: from diagnosis to treatment and the science in between. Cancer-Am Cancer Soc 2016; 122: 2299-2312.

16) Johansson P, Aoude LG, Wadt K, Glasson WJ, Warrier SK, Hewitt AW, Kiilgaard JF, Heegaard S, Isaacs T, Franchina M, Ingvar C, Vermeulen T, Whitehead KJ, Schmidt CW, Palmer JM, Symmons J, Gerdes AM, Jonsson G, Hayward NK. Deep sequencing of uveal melanoma identifies a recurrent mutation in PLCB4. Oncotarget 2016; 7: 4624-4631.

17) Algazi AP, Tsai KK, Shoushtari AN, Munhoz RR, Eroglu Z, Piulats JM, Ott PA, Johnson DB, Hwang J, Daud AI, Sosman JA, Carvajal RD, Chmielowski B, Postow MA, Weber JS, Sullivan RJ. Clinical outcomes in metastatic uveal melanoma treated with PD-1 and PD-L1 antibodies. Cancer 2016; 122: 3344-3353.

18) Ciarapica R, De Salvo M, Carcarino E, Bracaglia G, Adesso L, Leoncini PP, Dall'Agnese A, Walters ZS, Verginelli F, De Sio L, Boldrini R, Inserra A, Bisogno G, Rosolen A, Alaggio R, Ferrari A, Collini P, Locatelli M, Stifani S, Screpanti I, Rutella S, Yu Q, Marquez VE, Shipley J, Valente S, Mai A, Miele L, Puri PL, Locatelli F, Palacios D, Rota R. The polycomb group (PcG) protein EZH2 supports the survival of PAX3-FOXO1 alveolar rhabdomyosarcoma by repressing FBXO32 (Atrogin1/MAFbx). Oncogene 2014; 33: 4173-4184.

19) Qin H, Zhang G, Zhang L. GSK126 (EZH2 inhibitor) interferes with ultraviolet A radiation-induced photoaging of human skin fibroblast cells. Exp Ther Med 2018; 15: 3439-3448.

20) Kim KH, Roberts CW. Targeting EZH2 in cancer. Nat Med 2016; 22: 128-134.

21) Dardenne E, Beltran H, Benelli M, Gayvert K, Berger A, Puca L, Cyrta J, Sboner A, Noorzad Z, MacDonald T, Cheung C, Yuen KS, Gao D, Chen Y, Eilers M, Mosquera JM, Robinson BD, Elemento O, Rubin MA, Demichelis F, Rickman DS. N-Myc induces an EZH2-mediated transcriptional program driving neuroendocrine prostate cancer. Cancer Cell 2016; 30: 563-577.

22) Jiang T, Wang Y, Zhou F, Gao G, Ren S, Zhou C. Prognostic value of high EZH2 expression in patients with different types of cancer: a systematic review with meta-analysis. Oncotarget 2016; 7: 4584-4597.

23) Zhang ZZ, Zhao G, Zhuang C, Shen YY, Zhao WY, Xu J, Wang M, Wang CJ, Tu L, Cao H, Zhang ZG. Long non-coding RNA LINC00628 functions as a gastric cancer suppressor via long-range modulating the expression of cell cycle related genes. Sci Rep 2016; 6: 27435.

24) Kikuchi J, Koyama D, Wada T, Izumi T, Hofgaard PO, Bogen B, Furukawa Y. Phosphorylation-mediated EZH2 inactivation promotes drug resistance in multiple myeloma. J Clin Invest 2015; 125: 4375-4390.

25) Mallen-St Clair J, Soydaner-Azeloglu R, Lee KE, Taylor L, Livanos A, Pylayeva-Gupta Y, Miller G, Margueron R, Reinberg D, Bar-Sagi D. EZH2 couples pancreatic regeneration to neoplastic progression. Genes Dev 2012; 26: 439-444.