FULL LENGTH ARTICLE

Silencing of PRR11 suppresses cell proliferation and induces autophagy in NSCLC cells

Lian Zhang [a,b], Yunlong Lei [a,b], Ying Zhang [a,b], Yi Li [a,b], Youquan Bu [a,b], Fangzhou Song [a,b,**], Chundong Zhang [a,b,*]

[a] *Department of Biochemistry and Molecular Biology, Chongqing Medical University, 1 Yixueyuan Road, Yuzhong District, Chongqing, 400016, China*
[b] *Molecular Medicine and Cancer Research Center, Chongqing Medical University, 1 Yixueyuan Road, Yuzhong District, Chongqing, 400016, China*

Abstract Our previous studies have demonstrated that proline-rich protein 11 (PRR11) is a novel tumor-related gene and implicates in regulating the proliferation in lung cancer. However, its precise role in cell cycle progression remains unclear. Our recent evidences show that PRR11 silencing has an effect on autophagy in non-small-cell lung cancer (NSCLC) cells. Two human NSCLC cell lines, H1299 and A549 were transiently transfected with against PRR11 siRNA. The Cell Counting Kit-8 and plate clone formation assay showed that downregulation of PRR11 inhibited the cell proliferation associated with cell cycle related genes reduced. And our data suggested that PRR11 depletion expression enhanced the autophagosomes formation, followed with downregulation of P62 and upregulation of LC3-II protein. Furthermore, the immunoblotting results indicated that silencing of PRR11 inactivated the Akt/mTOR signaling pathway. Collectively, these results demonstrated PRR11 had an effective role in autophagy in NSCLC cells through Akt/mTOR autophagy signaling pathways. Therefore, it is helpful to clarify the function of PRR11 in tumorigenesis of NSCLC.

Introduction

Lung cancer is the most cause of worldwide cancer-related mortality, resulting in over a million deaths every year.[1,2] Lung cancer is mainly classified into small cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC) by tissue subtypes. NSCLC accounts for ~80% of lung cancer, including large cell carcinoma, adenocarcinoma and squamous cell carcinoma.[3] To date, surgical resection combined with radiotherapy and chemotherapy remains the primary methods of clinical treatment for lung cancer. However, up to 70% of NSCLC patients are diagnosed with advanced-stage disease.[4] Besides, the different clinical presentation of NSCLC patients can be caused by diverse molecular mechanisms that drive malignant transformation and dissemination of the primary tumor. Although there have been advance in NSCLC treatment, the patients still have poor prognosis and five-year survival rate is ~15%.[5] Therefore, it is helpful and beneficial to understand the biology of lung cancer in the clinical therapy and prognosis of malignant tumors.

Autophagy is an evolutionarily conserved self-degradation pathway, in which cell's components is sequestered in double-membrane vesicles and then delivered to the lysosome for degradation.[6,7] Under basal conditions, autophagy is a critical cellular homeostatic mechanism with stress resistance and pro-tumor or anti-tumor effects et al[8-10] Except for these, the most eye-catching function of autophagy is the role in cancer, which is dynamic and highly complex but not immutable. On the one hand, basal autophagy plays a role of a tumor suppressor by maintaining genomic stability in normal cells. On the other hand, once a tumor is established, down-regulated autophagy will contribute to carcinoma cell survival under tumor microenvironment and facilitate tumor growth and development.[11] The dynamic role of autophagy can also apply to lung carcinoma. Silencing or over-expression of autophagic crucial genes such as ATG5 or Beclin 1 acts a key role in the occurrence and development of NSCLC although the exact molecular mechanisms remain highly controversial. Diverse signaling pathways involving in autophagy, such as ERK/MAPK pathway and Akt/mTOR pathway et al, occupy an important position in the complex role of autophagy in NSCLC.[12,13]

Our previous studies demonstrated that PRR11 is implicated in lung cancer development and cell cycle progression. Silencing and overexpression of PRR11 led to a remarkable growth retardation in cancer cells resulting from a cell cycle arrest. In addition, PRR11 knockdown induced the dysregulation of multiple genes involved in cell cycle, such as CCNA1, CCNA2 and CDK6.[14,15] However, the precise molecular mechanism behind PRR11-mediated regulation of cell cycle and tumorigenesis remained unclear. Previous studies demonstrated that autophagy is strongly associated with stress-related cell cycle responses. We therefore investigated whether PRR11 correlated with autophagy in NSCLC cells. We demonstrated that down-regulation of PRR11 significantly induced autophagy via Akt/mTOR signaling pathway in NSCLC cells, suggesting that PRR11 is a critical regulator of tumorigenesis through regulating these cellular processes.

Material and method

Cell culture

Human non-small lung carcinoma-derived H1299 and A549 cells were cultured in RPMI 1640 medium and Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS, GIBCO) and penicillin (100 IU/ml)/streptomycin (100 mg/ml), respectively. Cells were maintained at 37 °C in a water-saturated atmosphere of 5% CO_2 in air. For the detection of mycoplasma in Cell Culture used MYCOPLASMA STAIN KIT (Mpbio, California, USA).

siRNA-mediated knockdown

The nucleotide sequences of control siRNA and siRNA against PRR11 or ATG5 were described previously.[14-16] Prior to transfection, cells were seeded at a density of 5×10^4 cells/24-well tissue culture plate or 2×10^5 cells/6-well tissue culture plate and allowed to attach overnight. The indicated siRNAs were then transiently transfected into cells using Lipofectamine RNAiMAX transfection reagent (Invitrogen) according to the manufacturer's instructions.

RNA isolation and quantitative real-time PCR analysis

Total RNA was prepared using Total RNA Kit I (Omega Bio-Tek) according to the manufacturer's instructions, and reverse transcription of 1 μg of total RNA was carried out using random primers and PrimeScript (Takara) following the manufacturer's instructions. The resultant cDNA was amplified by quantitative real-time PCR using SYBR Premix Ex Taq™ (Takara) according to the manufacturer's recommendations. The relative expression level of the target gene compared with that of the housekeeping gene, GAPDH, was calculated by the $2^{-\Delta\Delta^e}$ method.[14,15] The expression of PRR11 was detected as previously described.[14] The primer sequences were CDK6 (forward 5′-GCGCCTATGGGAAGGTGTTC-3′ and reverse 5′-TTGGGGTGCTCGAAGGTCT-3′), CCNE (forward 5′-GTCACATACGCCAAACTGG-3′ and reverse 5′-TTTCTTGAG-CAACACCCT-3′), CCNA1 (forward 5′-GCGGATCCTTGCCT-GAGTGAGC-3′ and reverse 5′-GCGAATTCGCAGAAGCCTATGA-3′), CCNA2 (forward 5′-AATCAGTTTCTTACCCAATAC-3′ and reverse 5′-CTGATGGCAAATACTTGA-3′), and CCNB2 (forward 5′-GCGTTGGCATTATGGATCG-3′ and reverse 5′-TCTTCCGGGAAACTGGCTG-3′).

Measurement of cell viability

The cell proliferation was determined using Cell Counting Kit-8 (CCK-8) kit. In brief, the transient transfection H1299 and A549 cells with siControl or siPRR11, and collaboration with siAtg5 were plated at a density of 1×10^4 cells/well in 96-well multiplates. After 24 h, 10 μL of CCK-8 solution was added to each well and further incubated for 2 h. Then, the absorbance values were detected at a wavelength of 450 nm using a Bio-Rad microplate reader. The cell viability

was calculated by the optical density (OD) values of treated groups/OD values of control groups × 100%.

Antibodies and reagents

Chloroquine were obtained from Sigma—Aldrich. Primary antibodies against the following proteins were used in this study: phosphorylated and total forms of Akt and mTOR, Beclin 1 and LC3 were purchased from Cell Signaling Technology; LAMP 1 from Santa Cruz Biotechnology (Santa Cruz, CA), ATG5 from ABGENT, p62 from Bethyl; and monoclonal anti-BrdU antibody from Roche Applied Science. CDK6, CCNE, CCNA1, CCNA2 and CCNB2 from Abcam. To confirm equal loading, membranes were reproved with an anti-GAPDH antibody (Hangzhou Goodhere).

Indirect immunofluorescent staining

Cells were fixed and incubated with primary antibodies, followed by the incubation with Alexa 488/594-conjugated secondary antibodies. Cells were then mounted with medium containing 4′6-diamidino-2-phenylindole (DAPI, Sigma), and the preparations were visualized with a Leica fluorescence microscope and a Zeiss confocal LSM 768 microscope.

BrdU labeling assay The BrdU labeling assay was performed in 24-well plate using the BrdU Cell Proliferation Assay Kit (Roche). After PRR11 siRNA treatment, BrdU was added to each well, and the cells were incubated for 3 h at 37 °C.

Immunoblotting analysis

Cells were lysed in RIPA lysis buffer (Beyotime) supplemented with protease inhibitor mixture (Beyotime). Protein concentrations of the lysates were determined by BCA reagent (Applygen Technologies). Equal amounts of the lysates (30 μg of protein) were denatured at 100 °C for 5 min, separated by 10% standard SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and electro-transferred onto polyvinylidene difluoride membranes (Millipore). The membranes were blocked with 5% non-fat dry milk in Tris-buffered saline (TBS) containing 0.1% Tween 20 at 4 °C overnight. After blocking, the membranes were then probed with the indicated primary antibodies at room temperature for 1 h, followed by the incubation with the corresponding horseradish peroxidase (HRP)—conjugated secondary antibodies at room temperature for 1 h. The proteins were finally visualized by enhanced chemiluminescence (ECL, Amersham).

Transmission electron microscopy

Transmission electron microscopy was performed as described previously.[17] Briefly, H1299 cells were fixed in 4% glutaraldehyde (Sigma). A sorvall MT5000 microtome (DuPont Instruments, MT5000) was used to prepare ultrathin sections after dehydration. Lead citrate and/or 1% uranyl acetate were used to stain the sections, and the

autophagic vacuoles in the cytoplasmic area were calculated using Image Pro Plus version 3 software.

Statistical analyses

Statistical evaluations were performed with GraphPad software (www.graphpad.com), and results were shown as mean ± SD unless otherwise stated. Statistical significance was set at a p value of <0.05, and marked with an asterisk.

Results

PRR11 silencing inhibits cell viability in NSCLC cells

Our previous studies demonstrated that PRR11 is related to cell cycle progression of lung cancer cells.[14,15] To further characterize the role of PRR11 in NSCLC, we first determined whether depletion of PRR11 affected cell growth in H1299 and A549 cells. Forty-eight hours after transfection, total RNA and whole cell lysates were prepared and then subjected to quantitative real-time PCR and immunoblotting analysis, respectively. The expression of PRR11 was significantly reduced at both mRNA and protein levels under our experimental conditions (Fig. 1A). Our recent studies suggested that silencing of PRR11 caused a visible cell cycle arrest.[15] CCK8 analysis showed that PRR11 depletion decreased the cell viability compared with control groups in both H1299 and A549 cell lines (Fig. 1B). As shown in Fig. 1C, the results from colony formation assays further confirmed that PRR11 depletion inhibited the growth of A549 and H1299 Cells. Moreover, the number of BrdU-positive cells in PRR11-depletion cells was significantly fewer than that of BrdU-positive cells in the control group (more than 600 positive-cells were counted, respectively) (see Fig. 1D). Consistently, PRR11 knockdown induced the reduction of multiple genes involved in cell cycle, such as CDK6, CCNE, CCNA1, CCNA2 and CCNB2 (Fig. 1E). As shown in Fig. 1F, the flow cytometry assessments demonstrated that depletion of PRR11 also induced a little apoptosis in H1299 and A549, but the low apoptosis ratio could not significantly affect cell proliferation. Taken together, these data demonstrate that silencing of PRR11 expression could remarkably inhibit the viability as well as a few apoptosis of NSCLC cells.

Silencing of PRR11 expression stimulates autophagy in NSCLC cells

Reports have demonstrated a close correlation between autophagy and cell-cycle responses,[18] we next investigated whether silencing of PRR11 expression could regulate autophagy in NSCLC cells. We first estimated the effect of PRR11 depletion expression on the formation of autophagosome membrane by analyzing two classical markers of autophagy: a fraction of the LC3-I into LC3-II, and the distribution of endogenous LC3 puncta.[19] As shown in Fig. 2A and B, silencing of PRR11 resulted in remarkably induced autophagy as evidenced by high level of LC3-II expression and increased LC3 puncta. In addition, the expression levels
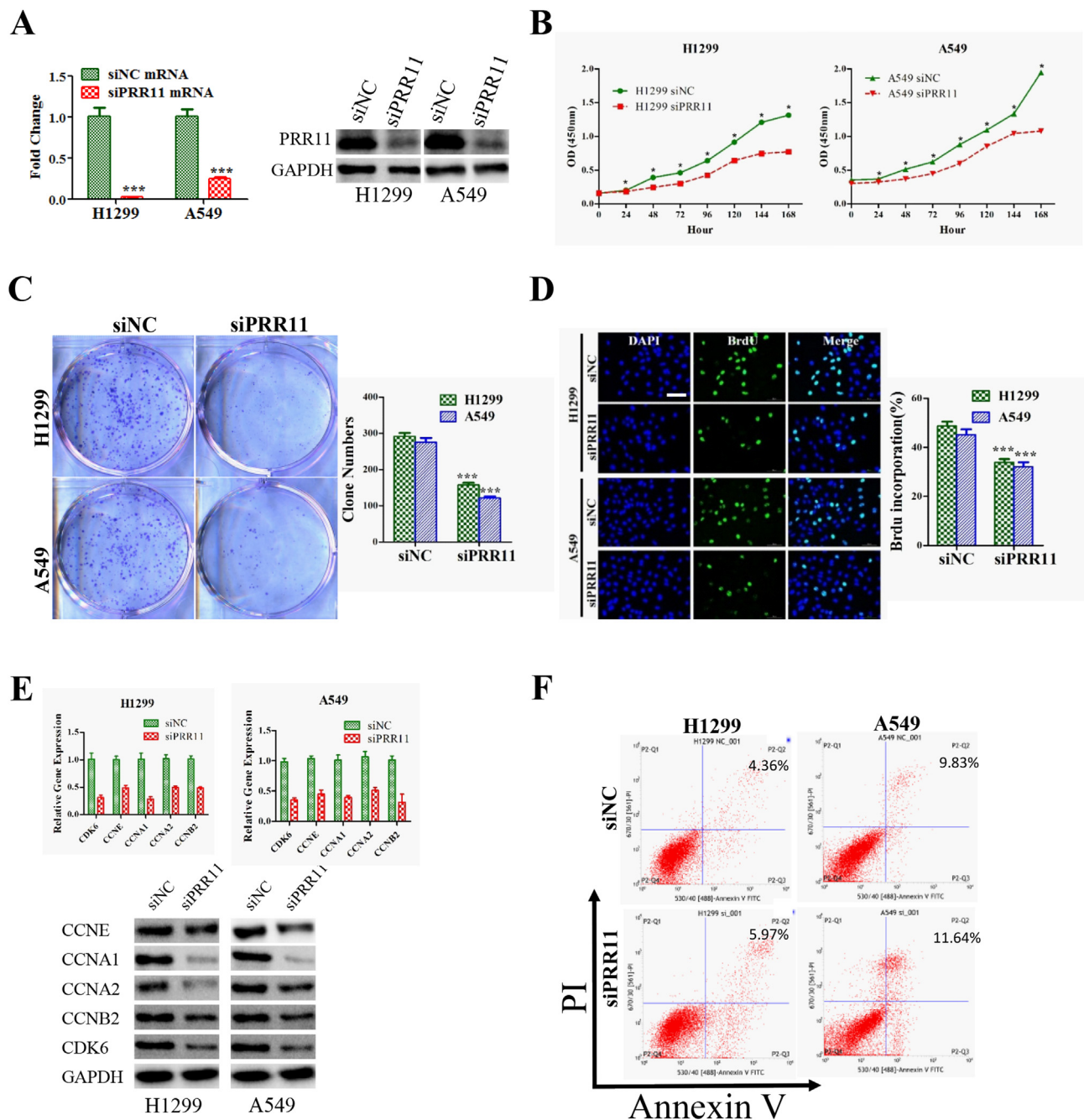
Figure 1 Silencing of PRR11 inhibits cell viability in NSCLC cells. (A) siRNA-mediated silencing of PRR11. H1299 and A549 cells were transiently transfected with a negative control siRNA (NC siRNA) or with siRNA against PRR11. Forty-eight hours after transfection, total RNA and whole cell lysates were prepared and subjected to RT-PCR (left) and immunoblotting (right), respectively (B) The effect of PRR11 depletion expression with the cellular proliferation. Cells as siNC and siPRR11 treatment was determined by CCK8 assay at indicated timepoints. *,$p < 0.01$, **,$p < 0.001$ (C) Silencing of PRR11 expression suppressed colony formation in lung cancer cells. Cells were cultured for 8 days (D) Depletion of PRR11 expression inhibited lung cancer cells proliferation measured BrdU labeling. Scale bars, 50 μm***,$p < 0.0001$ (E) H1299 and A549 cells were transiently transfected with a negative control siRNA (NC siRNA) or with siRNA against PRR11. Forty-eight hours after transfection, total RNA and whole cell lysates were prepared, and RT-PCR (above) and immunoblotting (under) was used to determine the expression levels of indicated genes, respectively. GAPDH was used as an internal control (F) Cell apoptosis analysis in H1299 and A549 cells. Cells were transiently transfected with siRNA. Forty-eight hours after transfection, attached and suspension cells were harvested, and then the apoptosis were analyzed by FACS.
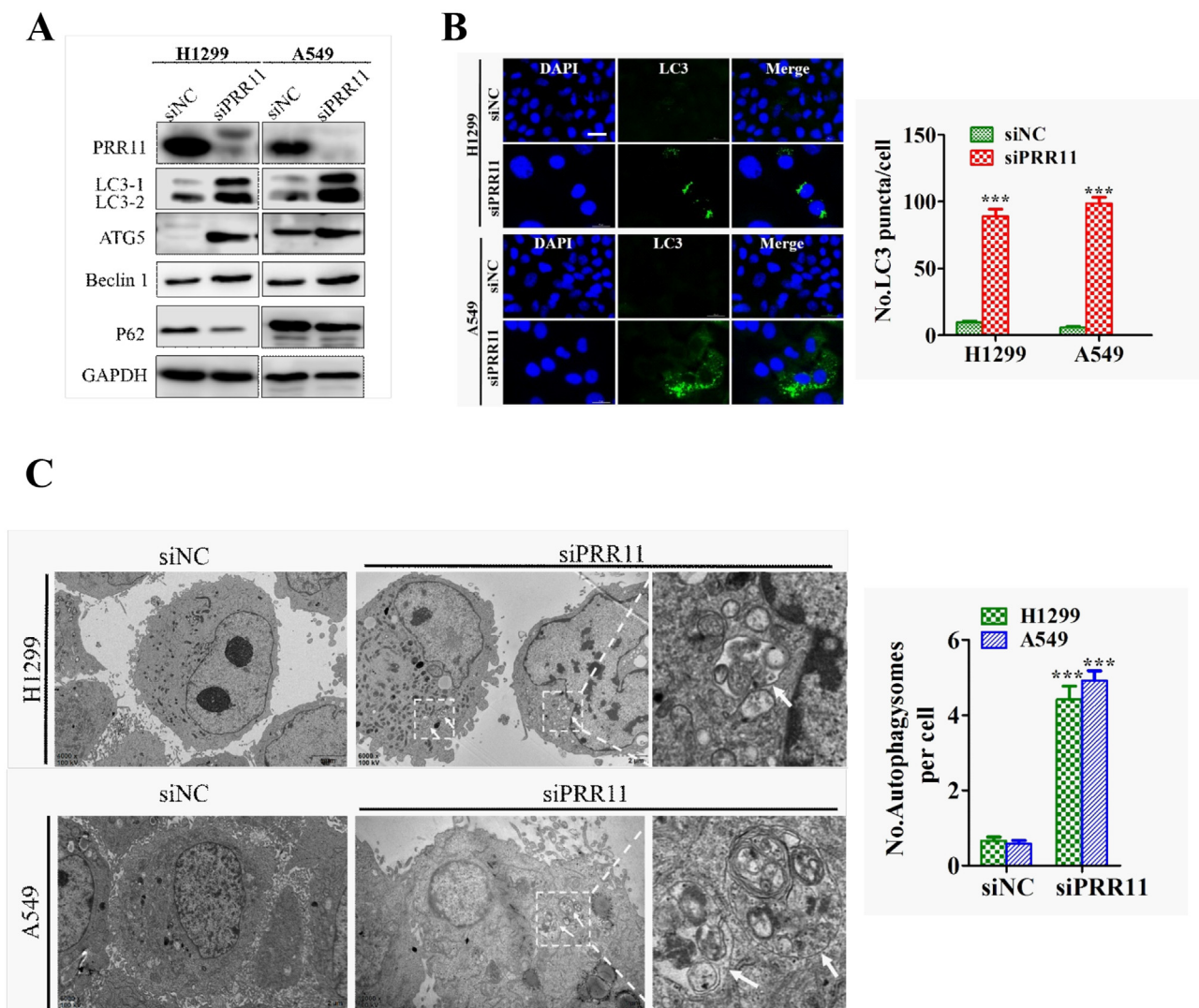
Figure 2 Silencing of PRR11 stimulates autophagy in NSCLC cells. (A) Immunoblot analysis expression of LC3, Atg5, Beclin1 and p62 in H1299 and A549 cells. Cells were transiently transfected with a negative control siRNA (NC siRNA) or with siRNA against PRR11. Forty-eight hours after transfection, whole cell lysates were prepared and subjected to immunoblotting as indicated proteins (B) PRR11 depletion expression promotes autophagy. Left, Representative images of the formation of endogenous LC3 puncta in cells treated with PRR11 siNC or siRNA for 48 h. Right, total number of endogenous LC3 puncta per cells. Scale bars, 20 μm (C) Autophagy measured by transmission electron microscopy in cells treated with as in (B). Arrows, autophagosomes/autolysomes. Right, total number of autophagosomes per cell. ***, $p < 0.0001$. Scale bars, 1 μm.

of two autophagy-related proteins Atg5 and Beclin 1,[19] were examined to further clarify whether depletion of PRR11 expression promoted autophagosome formation. Results demonstrated that PRR11 depletion promoted the expression of both Beclin 1 and Atg5 (Fig. 2A). Moreover, silencing of PRR11 expression resulted in low level of p62 expression, a well-known autophagic substrate (Fig. 2A). Finally, to further explore silencing of PRR11 expression induced autophagy, the appearance of double-membraned autophagic vesicles (autophagosomes) was analyzed by transmission electronic microscopy. The results stated a significant accumulation of autophagosomes/autolysosomes in PRR11 depletion cells but not in control cells (Fig. 2C). Together, these data indicate that silencing of PRR11 expression stimulates autophagy in NSCLC cells.

Silencing of PRR11 expression promotes autophagy flux in NSCLC cells

In order to study the role of PRR11 depletion in the autophagic process including autophagosome formation, fusion with lysosome and degradation in autolysosome in NSCLC cells, autophagosomes were stained with a specific tandem monomeric RFP-GFP-tagged LC3,[19,20] and the number of yellow fluorescent autophagosomes and red fluorescent autolysosomes was identified (Fig. 3A and B). Consistently, LC3 and lysosome-associated membrane protein 1 (Lamp1) double-positive autolysosomes accumulated at the extreme periphery of the cell, and exhibited relatively high intensity (Fig. 3C).
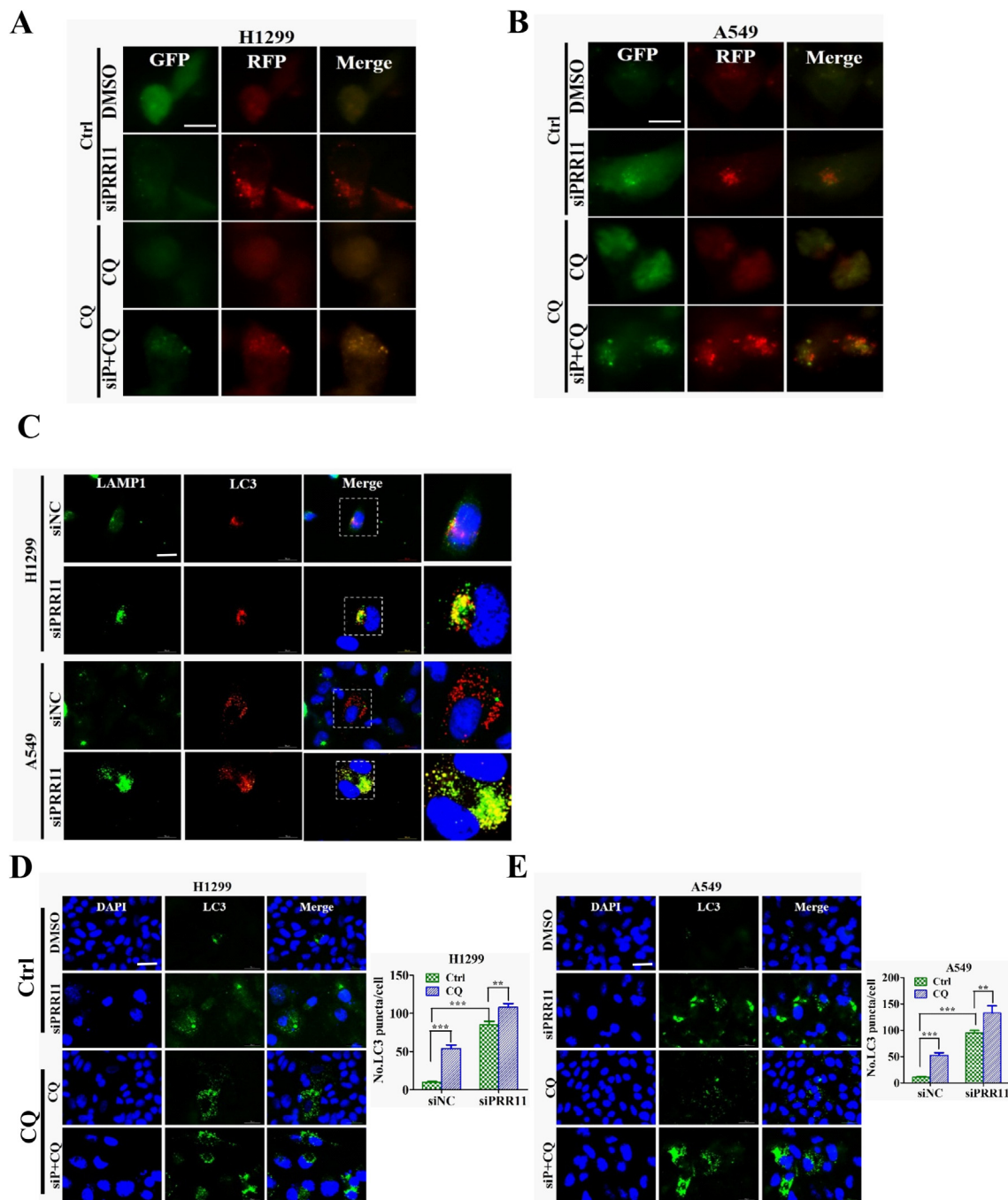
Figure 3 Depletion of PRR11 expression promotes autophagy flux in NSCLCs. (A) and (B) Cells were transiently transfected with an RFP-GFP-LC3 tandem fluorescent-tagged LC3 (RFP-GFP-LC3). In addition, cells were treated with PRR11 siRNA alone for 48 h or in combination with 10 μmol/L chloroquine (CQ) for 24 h. Scale bars, 10 μm (C) Immunofluorescent staining was performed for LAMP1 and analyzed by microscopy. Scale bars, 20 μm (D) and (E) Left, immunofluorescence analysis of endogenous LC3 puncta in H1299 and A549 cells. Scale bars, 20 μm ***, $p < 0.0001$. Scale bars, 20 μm.

To further examine the changes in the autophagic flux, PRR11 silencing was combined with the lysosomotropic agent chloroquine which inhibits both the fusion of autophagosome with lysosome and lysosomal protein degradation. The increased number of yellow fluorescent autophagosomes and endogenous LC3 puncta was detected in PRR11 depletion cells treated with chloroquine (Fig. 3D—E). Altogether, these results indicate that silencing of PRR11 expression induces autophagic flux in lung cancer cells.

Autophagy is involved in inhibiting cell proliferation by PRR11 silencing in NSCLC cells

To test whether autophagy was involved in the proliferation-inhibition of PRR11 depletion expression in

NSCLC cells, cells were transfected with PRR11 siRNA combination with Atg5 siRNA. The expression of Atg 5 was significantly reduced at protein levels under our experimental conditions (Fig. 4A). Cell viability was assessed by CCK8 assay, BrdU labeling, and colony formation analysis. As shown in Fig. 4B–C, Cell growth was decreased by a combination treatment of PRR11 and Atg5 siRNA. Consistently, the number of BrdU-positive cells in combinatorial treatment with Atg5 siRNA group was predominantly smaller than that in group only treated with PRR11 siRNA (More than 600 positive-cells were counted, respectively) (Fig. 4D). Thus, these findings suggest that silencing of
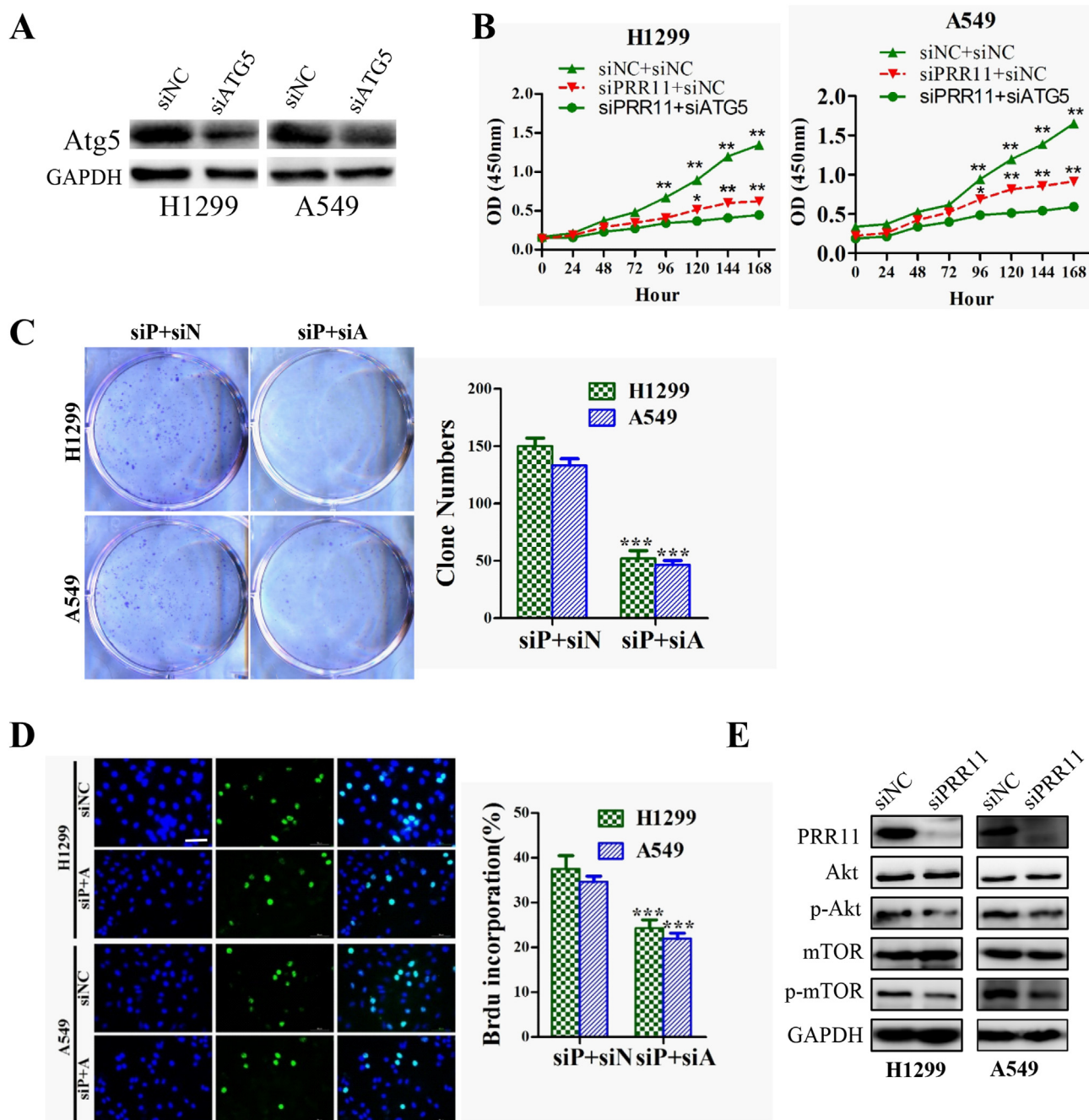

Figure 4 Autophagy is involved in inhibiting cell proliferation by PRR11 silencing in NSCLC cells through Akt/mTOR signaling pathway. (A) Cells were transiently transfected with a negative control siRNA (NC siRNA) or with siRNA against ATG5. Forty-eight hours after transfection, whole cell lysates were prepared and subjected to immunoblotting in H1299 and A549 cells. Cells were transfected with Atg5 siRNA in combination with PRR11 siRNA. Cellular proliferation was detected by CCK8 assay (B), colony formation (C) and BrdU labeling (D). Scale bars, 50 μm *, $p < 0.01$,**, $p < 0.001$, ***, $p < 0.0001$ (E) Immunoblot analysis of phosphorylation of Akt (S473) and mTOR (S2448) in cells treated with PRR11 siRNA for 72 h. Total Akt and mTOR was used as the internal control, respectively.

PRR11 expression activates autophagy as a survival mechanism for stress, and suppression of autophagy enhances effect of proliferation-inhibition mediated by PRR11 depletion expression in NSCLC cells.

PRR11 silencing induces autophagy through Akt/mTOR signaling pathway

It has been reported that constitutively activated Akt/mTOR signaling was involved in regulating cell cycle and autophagy, and Akt/mTOR acted as a key negative modulator in autophagy.[21] Therefore, to determine whether the proliferation inhibition caused by PRR11 depletion expression was related to this pathway in NSCLC cells, we investigated the expression level of the representative Akt/mTOR signal proteins by PRR11 depletion. As shown in Fig. 4E, silencing of PRR11 expression resulted in inhibition of Akt/mTOR pathway, as evidenced by decreased phosphorylation levels of Akt and mTOR, but had no effect on the expression levels of total Akt and mTOR. It suggests that Akt/mTOR pathway is an important mediator in silencing of PRR11 expression-induced autophagy.

Discussion

Our previous study identified PRR11 as a novel cancer-related gene involving in both cell cycle progression and lung cancer development.[14,15] Moreover, subsequent studies reported that PRR11 also had oncogenic potential and prognostic value in gastric cancer, hilar cholangiocarcinoma and pancreatic cancer. Up to now, there has been no data about PRR11 in association with autophagy and proliferation of cancer cell. In this study, we demonstrate that PRR11 silencing induces autophagy and inhibits proliferation in NSCLC cells and the Akt/mTOR signaling pathway is required for this autophagy.

Several studies have suggested that both mRNA and protein levels of PRR11 was overexpressed in lung cancer, gastric cancer, breast cancer hilar cholangiocarcinoma and pancreatic cancer.[14,22–24] Furthermore, PRR11 expression is closely associated with poor prognosis in cancer patients. In addition, our previous studies have demonstrated that PRR11 expression is oscillated in a cell cycle-dependent manner. During the cell cycle, the amount of PRR11 starts to accumulate in the late S phase, and is retained until before mitotic telophase. Consistently, silencing of PRR11 leads to late-S phase arrest and G2/M progression dysregulation. However, the molecular mechanism implicated in growth of human cancer cells has not been investigated. Our present studies have demonstrated that knockdown of PRR11 could effectively inhibit the proliferation of A549 and H1299 lung cancer cells. And then PRR11 knockdown induced the dysregulation of multiple genes involved in cell cycle, such as CDK6, CCNE, CCNA1, CCNA2 and CCNB2. Intriguingly, CCK8 and cell clone formation assay showed that proliferation inhibition effect was significantly enhanced in ATG5-and PRR11-depleted cells. Our results suggest that PRR11 may repress cell proliferation by inhibiting autophagy.

Autophagy contributes to the pathogenesis of cancer, and can act either as a tumor-suppressive or a tumor-promoting pathway.[7,25] Autophagy-deficient animal models, inducing DNA damage and chromosomal instability (CIN), are not subject to tumor formation.[26] Therefore, autophagy is helpful against malignant transformation by protecting intracellular homeostasis.[6,18,27,28] However, autophagy can also sustain the survival and proliferation of neoplastic cells exposed to intracellular and environmental stresses, and thereby facilitates tumor growth and progression.[18,29] The activation of Akt or mTOR is heavily implicated in the development of human cancer, including lung cancer.[8,30] Previous studies have demonstrated that the Akt/mTOR signaling pathway may repress autophagy in response to insulin-like and other growth factor signals.[6] The activation of Akt or mTOR is heavily implicated in the development of human cancer, including lung cancer. Previous studies have demonstrated that the Akt/mTOR signaling pathway may repress autophagy in response to insulin-like and other growth factor signals. The present study revealed that silencing of PRR11 may inactivate the Akt/mTOR signaling pathway and promote autophagy. Previous studies has demonstrated that inhibition of the Akt/mTOR signaling pathway is closely related to autophagy in non-small cell lung cancer cells.

In summary, to investigate the probable mechanism of anti-proliferative efficacy of PRR11 in NSCLC, we examined the effect of PRR11 knockdown on autophagy. In this study, we introduce PRR11 as a new autophagy regulatory gene implicated in cell cycle progression and tumorigenesis. Our results have shown that knockdown of PRR11 promotes protective autophagy in H1299 and A549 lung cancer cells. The results provide a better understanding of the mechanisms for the role of PRR11 in tumor development, and might serve as a potential target in the diagnosis and/or treatment of human lung cancer.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

References

1. Kong L, Zhang P, Li W, et al. KDM1A promotes tumor cell invasion by silencing TIMP3 in non-small cell lung cancer cells. *Oncotarget*. 2016;7:27959—27974.
2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA A Cancer J Clin*. 2013;63:11—30.
3. Chen Z, Fillmore CM, Hammerman PS, Kim CF, Wong KK. Non-small-cell lung cancers: a heterogeneous set of diseases. *Nat Rev Cancer*. 2014;14:535—546.
4. Bai H, Mao L, Wang HS, et al. Epidermal growth factor receptor mutations in plasma DNA samples predict tumor response in

Chinese patients with stages IIIB to IV non-small-cell lung cancer. *J Clin Oncol*. 2009;27:2653—2659.

5. Ettinger DS, Akerley W, Borghaei H, et al. Non-small cell lung cancer, version 2.2013. *J Natl Compr Canc Netw*. 2013;11: 645—653. quiz 653.

6. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell*. 2008;132:27—42.

7. Klionsky DJ, Emr SD. Autophagy as a regulated pathway of cellular degradation. *Science*. 2000;290:1717—1721.

8. White E, DiPaola RS. The double-edged sword of autophagy modulation in cancer. *Clin Cancer Res*. 2009;15: 5308—5316.

9. Mizushima N, Ohsumi Y, Yoshimori T. Autophagosome formation in mammalian cells. *Cell Struct Funct*. 2002;27:421—429.

10. Shintani T, Klionsky DJ. Autophagy in health and disease: a double-edged sword. *Science*. 2004;306:990—995.

11. White E. The role for autophagy in cancer. *J Clin Invest*. 2015; 125:42—46.

12. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol*. 2011;13:132—141.

13. Han Y, Wu Z, Wu T, et al. Tumor-suppressive function of long noncoding RNA MALAT1 in glioma cells by downregulation of MMP2 and inactivation of ERK/MAPK signaling. *Cell Death Dis*. 2016;7:e2123.

14. Ji Y, Xie M, Lan H, et al. PRR11 is a novel gene implicated in cell cycle progression and lung cancer. *Int J Biochem Cell Biol*. 2013;45:645—656.

15. Zhang C, Zhang Y, Li Y, et al. PRR11 regulates late-S to G2/M phase progression and induces premature chromatin condensation (PCC). *Biochem Biophys Res Commun*. 2015;458: 501—508.

16. Wang Y, Zhang C, Jin Y, et al. Alkaline ceramidase 2 is a novel direct target of p53 and induces autophagy and apoptosis through ROS generation. *Sci Rep*. 2017;7:44573.

17. Liu R, Li J, Zhang T, et al. Itraconazole suppresses the growth of glioblastoma through induction of autophagy: involvement of abnormal cholesterol trafficking. *Autophagy*. 2014;10: 1241—1255.

18. Mathiassen SG, De Zio D, Cecconi F. Autophagy and the cell cycle: a complex landscape. *Front Oncol*. 2017;7:51.

19. Klionsky DJ, Abdelmohsen K, Abe A, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (3rd ed.). *Autophagy*. 2016;12:1—222.

20. Dou Q, Chen HN, Wang K, et al. Ivermectin induces cytostatic autophagy by blocking the PAK1/Akt axis in breast cancer. *Cancer Res*. 2016;76:4457—4469.

21. Schmelzle T, Hall MN. TOR, a central controller of cell growth. *Cell*. 2000;103:253—262.

22. Chen Y, Cha Z, Fang W, et al. The prognostic potential and oncogenic effects of PRR11 expression in hilar cholangiocarcinoma. *Oncotarget*. 2015;6:20419—20433.

23. Song Z, Liu W, Xiao Y, et al. PRR11 is a prognostic marker and potential oncogene in patients with gastric cancer. *PLoS One*. 2015;10, e0128943.

24. Tan S, Jiang Z, Hou A, Wang J, Zhang J, Dai L. Expression of PRR11 protein and its correlation with pancreatic cancer and effect on survival. *Oncol Lett*. 2017;13:4117—4122.

25. Nakagawa I, Amano A, Mizushima N, et al. Autophagy defends cells against invading group a streptococcus. *Science*. 2004; 306:1037—1040.

26. Mathew R, Kongara S, Beaudoin B, et al. Autophagy suppresses tumor progression by limiting chromosomal instability. *Genes Dev*. 2007;21:1367—1381.

27. Galluzzi L, Pietrocola F, Bravo-San Pedro JM, et al. Autophagy in malignant transformation and cancer progression. *EMBO J*. 2015;34:856—880.

28. White E. Deconvoluting the context-dependent role for autophagy in cancer. *Nat Rev Cancer*. 2012;12:401—410.

29. Neufeld TP. Autophagy and cell growth—the yin and yang of nutrient responses. *J Cell Sci*. 2012;125:2359—2368.

30. Cooper JM, Ou YH, McMillan E, et al. TBK1 provides context-selective support of the activated AKT/mTOR pathway in lung cancer. *Cancer Res*. 2017;77(18):5077—5094.