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FULL LENGTH ARTICLE

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

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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.

Abbreviations: proline-rich protein 11, (PRR11); small cell lung cancer, (SCLC); non-small-cell lung cancer, (NSCLC); Cell Counting Kit-8, (CCK8); lysosome-associated membrane protein 1, (Lamp1); chromosomal instability, (CIN).

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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 × 104 cells/24-well tissue culture plate or 2 × 105 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

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