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Knockdown of LncRNA PVT1 inhibits tumorigenesis in non-small-cell lung cancer by regulating miR-497 expression

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ABSTRACT

Plasmacytoma variant translocation1 (PVT1) was reported to be upregulated in non-small-cell lung cancer (NSCLC) tissues, serve as a promising biomarker for diagnosis and prognosis of NSCLC, and promoted NSCLC cell proliferation. However, the detailed molecular mechanism of PVT1 involved in the pathogenesis and development of NSCLC remains largely unknown. In this study, the expression levels of PVT1 and miR-497 in NSCLC cells were determined by qRT-PCR. Cell viability, invasion and apoptosis were detected by MTT assay, cell invasion assay and flow cytometry analysis, respectively. RNA immunoprecipitation (RIP) and luciferase reporter assay were performed to confirm whether PVT1 directly interacts with miR-497. A xenograft mouse model was established to confirm the effect of PVT1 on tumor growth *in vivo* and the underlying molecular mechanism. Our findings indicated that PVT1 was significantly upregulated and miR-497 was markedly downregulated in NSCLC cell lines. si-PVT1 effectively decreased the expression of PVT1 and increased the expression of miR-497. PVT1 knockdown remarkably inhibited cell viability, invasion and promoted apoptosis in NSCLC cells. RIP and luciferase reporter assay demonstrated that PVT1 could directly interact with miR-497. Moreover, PVT1 overexpression reversed the inhibitory effect of miR-497 on cell viability, invasion and promotion effect on apoptosis of NSCLC cells. Furthermore, *in vivo* experiment showed that knockdown of PVT1 inhibited tumor growth *in vivo* and promoted miR-497 expression. In conclusion, knockdown of PVT1 inhibited cell viability, invasion and induced apoptosis in NSCLC by regulating miR-497 expression, elucidating the molecular mechanism of the oncogenic role of PVT1 in NSCLC and providing an lncRNA-directed target for NSCLC.

1. Introduction

Lung cancer is one of the most common type of cancers and a predominant cause of cancer-relevant death throughout the world, with an annual incidence of more than 226,000 new diagnosed cases [1]. Non-small-cell lung cancer (NSCLC), accounting for approximately 85% of all lung cancer cases, is generally diagnosed at an advanced stage, most of which are accompanied by advanced local invasion and/or distant metastasis [2]. In spite of considerable improvements in early diagnosis and treatment of NSCLC over the past years, the prognosis of lung cancer patients remains unsatisfactory, with the overall 5-year survival rate for patients still as low as 16.8% [3,4]. Increasing evidence has indicated that the pathogenesis and progression of NSCLC are a complicated biology process, which is attributed to dysregulation of multiple oncogenes and tumor-suppressive genes [5,6]. Hence, an improved and detailed understanding of the molecular mechanism underlying NSCLC development and progression is critical for improving the therapy and prognosis of NSCLC.

Recently, the rapid development of microarrays and high-throughput sequencing techniques revealed that the vast majority of the mammalian genome is transcribed into noncoding RNAs (ncRNAs), mainly including long ncRNAs (lncRNAs) and microRNAs (miRNAs) [7]. LncRNAs are a class of non-coding RNAs more than 200 nucleotides (nt) with no or limited protein-coding potential. It is well documented that lncRNAs play a functional role in a wide range of pathological and physiological process, such as cellular development, migration, invasion, and apoptosis, by regulating gene expression at the chromatin modification, transcriptional or post-transcriptional levels [8]. Emerging evidence suggests that the aberrant expressions of lncRNAs appear to be involved in the development and progression of various types of cancers including NSCLC [9–11]. The human plasmacytoma variant translocation1 (PVT1) oncogene is a lncRNA with 1716 nt in length that located adjacent to oncogene c-myc on chromosome 8q24.21 region [12]. Recent studies have shown that PVT1 was highly expressed and closely associated with the pathophysiology of a wide variety of cancers, including breast cancer, ovarian cancers and acute myeloid

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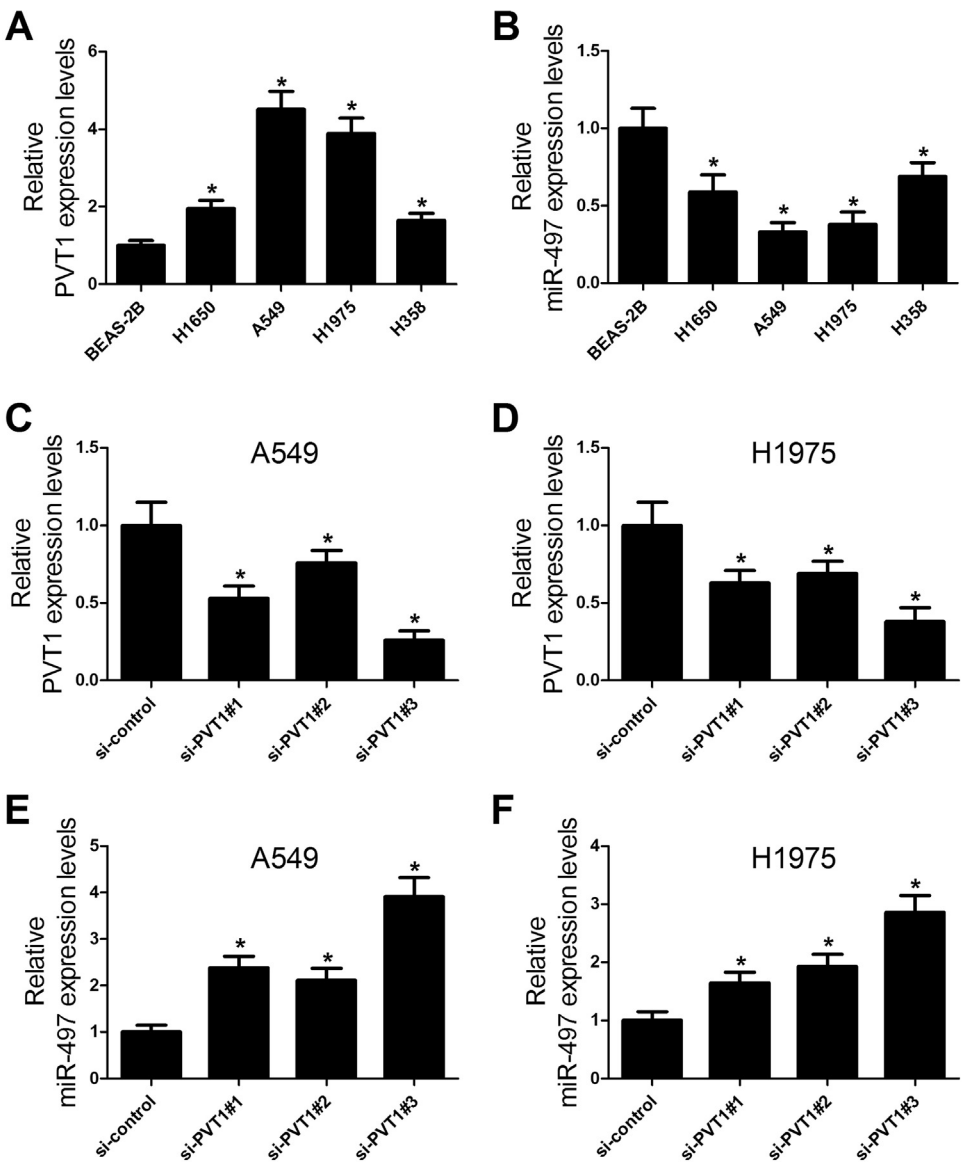

Fig. 1. Knockdown of PVT1 increased miR-497 expression in NSCLC cells. qRT-PCR analysis of the expressions of PVT1 (A) and miR-497 (B) in four NSCLC cell lines (H1650, A549, H1975, and H358) and normal human lung epithelial cells BEAS-2B. qRT-PCR analysis of the expressions of PVT1 in A549 (C) and H1975 (D) cells transfected with si-PVT1#1, si-PVT1#2, si-PVT1#3, or si-control. qRT-PCR analysis of the expressions of miR-497 in A549 (E) and H1975 (F) cells transfected with si-PVT1#1, si-PVT1#2, si-PVT1#3, or si-control. $^*P < 0.05$.

leukemia [13,14]. Additionally, PVT1 was reported to be upregulated in NSCLC tissues, serve as a promising biomarker for diagnosis and prognosis of NSCLC, and promote NSCLC cell proliferation [15,16]. However, the detailed molecular mechanism of PVT1 involved in the pathogenesis and development of NSCLC remains largely unknown.

miRNAs are small, endogenous ncRNAs with 22 nucleotides in length that play a crucial regulatory role in the expression of target gene by binding to complementary sites in 3′-untranslated regions (3′UTRs) of the target mRNA [17]. An increasing number of studies reveal that dysregulation of multiple miRNAs is closely associated with the development of human lung cancer by functioning as oncogenes or tumor suppressors [18]. Recently, the competing endogenous RNA (ceRNA) regulatory network proposed that a large number of lncRNAs might function as molecular sponges or antagomirs for miRNAs and, thereby, regulate the expression and biological activity of miRNAs in cancer progression [19]. For example, lncRNA colon cancer-associated transcript 1 (CCAT1) was revealed to play a pivotal role in hepatocellular carcinoma (HCC) progression via functioning as let-7 sponge [20]. In gallbladder carcinoma (GBC) cells, lncRNA taurine-upregulated gene 1 (TUG1) promoted cell proliferation, metastasis, and epithelial-mesenchymal transition (EMT) progression by acting as a miR-300 sponge [21]. Acting as a miR-206 sponge, lncRNA mitochondrial RNA processing endoribonuclease (RMRP) promoted carcinogenesis of gastric cancer and may be used as a novel biomarker for gastric cancer [22]. Previous studies revealed that miR-497 was significantly downregulated in NSCLC tissues and inhibited tumor cell growth and invasion in NSCLC [23–25]. However, whether PVT1 could interact with miR-497 to participate in the pathogenesis of NSCLC remains to be elaborated.

In the present study, we aimed to explore the relationship between PVT1 and miR-497 and investigate the effects of their interaction on cell proliferation, invasion and apoptosis in NSCLC cells.

2. Materials and methods

2.1. Cell culture and transfection

NSCLC cell lines (H1650, A549, H1975, and H358) and normal human lung epithelial cells BEAS-2B were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). All cell lines were routinely cultured in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 100 μg/ml penicillin sodium, and 100 μg/ml streptomycin sulfate at 37 °C in a