Lung carcinoma is currently the most common malignancy in the world [1]. It is reported that morbidity and mortality of lung carcinoma have increased year by year, which have attracted widespread attention [1]. Advanced lung cancer is difficult to cure [2], and chemotherapy is considered as one of the main treatment methods [3]. Nowadays, the goals for lung carcinoma treatment are to reduce the symptoms, to improve the quality of life as well as to prolong life-span [3]. Therefore, it is imminent to develop novel and effective methods for lung cancer therapeutics. More importantly, the pathogenesis of lung carcinoma is complicated and has not yet been completely understood [4]. It is believed that it is associated with multiple oncogene activation and tumor suppressor gene inactivation [4].

MicroRNAs (miRNAs) are a class of non-coding single-stranded RNAs that are highly conserved in organisms [5], which have shown to be positively related to tumorigenesis [6]. In human tumors, expression levels of miRNAs were abnormal and several miRNAs were reported to be mutated [7]. miRNAs regulate cell growth, proliferation and migration by modulating the expression of tumor suppressor genes or oncogenes [8]. A large number of studies have demonstrated that miR-377 functions as a tumor suppressor gene in gastric cancer [9], ovarian cancer [10], prostate cancer [11] and non-small cell lung cancer (NSCLC) [12]. miR-377-5p is briefly mentioned in the tumor necrosis factor-receptor associated periodic syndrome (TRAPS), and its molecule mechanisms in lung cancer remained unclear [13].

Composed of 480 amino acids, protein kinase B (also known as AKT) functions as an intersection of multiple signaling pathways [14]. It plays a key role in cell cycle

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regulation, development as well as apoptosis [14, 15]. AKT could be activated upon phosphorylated phosphoinositide 3-kinase (PI3K) dependent kinase 1 and 2, and then participated in the regulation of cell growth and metabolism by activating target genes, such as mammalian target of rapamycin (mTOR), Bcl-xL/Bcl-2 associated death promoter (Bad), Bcl-2 associated X protein (Bax) and forkhead box protein O (Foxo) [16]. Interestingly, AKT1 is one of the important subtypes of AKT, which not only affects cell proliferation and apoptosis, but also regulates many tumor cell migration and invasion pathways [17]. These evidences suggested that AKT1 could be considered as a valuable therapeutic target. To date, several miRNAs have been reported to inhibit cell metastasis by targeting AKT1 in cancers [18, 19]. However, the effects and regulation mechanisms between miR-377 and AKT1 has not been elucidated. To investigate the effects of miR-377-5p and/or AKT1 on the development, invasion and metastasis of lung carcinoma, we measured their expressions in cancer tissues. Then, we studied molecular mechanisms of miR-377-5p, AKT1 and other related target genes of miR-377-5p affecting lung carcinoma cells.

For proliferation assay, cells were fixed by 4% paraformaldehyde (PFA, Solarbio, Beijing, China) for 20 min, washed twice in distilled water for 2 min, stained in crystal violet solution for 25 min and then cells were observed and photographed by a microscope (Leica Microsystems, Weitzlar, Germany).

Cell migration and invasion assay

Cells were cultured in 24-well plates, washed by 1xPBS and re-suspended in serum-free medium. Cell suspension (200 μl) was added to the upper of 8 μm

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transwell chamber with 0.1% bovine serum albumin (BSA, Saibao, Yancheng, China), while 500 µl medium with 10% FBS added to the lower of Transwell chamber containing fibronectin (Sigma-Aldrich, St. Louis, USA). Cells were incubated for 24 hrs at 37°C, stained by 10% crystal violet solution. And then cells observed and photographed using a microscope (Leica Microsystems, Weitzlar, Germany).

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Cell cycle assay

Cells were cultured in 6-plates (1.5 × 106 cells), digested with trypsin (Sigma-Aldrich, St. Louis, USA), re-suspended in 1xPBS and fixed in pre-cooled anhydrous ethanol overnight at 4°C. Then, fixed cells were washed by 1xPBS twice, 20 µl RNase were added (Sigma-Aldrich, St. Louis, USA) for 30 min at 37°C, then stained in 20 µl Propidium Iodide (PI, Sigma-Aldrich, St. Louis, USA) for 30 min at 4°C without light and detected using a Flow Cytometer (Thermo Fisher Scientific, Waltham, USA).

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Quantitative real time polymerase chain reaction (qRT-PCR)

Total RNAs of cells were extracted using RNAeasy™ RNA Isolation Kit with Spin Column (Beyotime, Shanghai, China). The concentration and purity of RNA were measured at 280 nm using spectrophotometer. RNA was reversely transcripted to cDNA using BeyoRT™ First Strand cDNA Synthesis Kit (Beyotime, Shanghai, China). All cDNA samples were equipped with PCR reaction system. The prepared PCR reaction solution was amplified using real-time fluorescent quantitative PCR (Thermo Fisher Scientific, Waltham, USA), and the reaction conditions were set up as follow: 93°C for 1 min, 55°C for 1 min, 72°C for 1 min, a total of 40 cycles. PCR products were detected in 2% agarose gel electrophoresis. The results were analyzed

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