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KIAA0101 Silencing Overcomes Cisplatin Resistance in Non-Small Cell Lung Cancer by Inhibiting PI3K/AKT/mTOR Pathway

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Abstract

We constructed KIAA0101 overexpression plasmids and KIAA0101 interference plasmids. MTT assay was used to detect the effect of KIAA0101 knockdown and overexpression on NSCLC cell resistance to cisplatin. Finally, we

formazan was dissolved in dimethyl sulfoxide (DMSO; Sigma) and the optical density (OD) values were detected under 570 nm wavelength to calculate the inhibition rate of cells. The calculation formula was as follows: cell inhibition rate (%) = 1 − (OD value of experimental group/OD value of normal group.

A 549, NCI-H520 and NCI-H1299 cells were seeded in 10cm plates at 2×10^6 cells and maintained for 24 h. We were culture with different concentrations of DDP (0, 5, 10, 20 μM; Sigma, USA) for 48h. Collect cells and extract proteins, the total protein was isolated using RIPA lysis buffer. The concentration of protein was determined using the bicinchoninic acid method. Equal amounts of proteins were separated by 10% SDS-PAGE to electrotransfer onto PVDF membranes. After sealing with 5% skim dried milk at room temperature, the membranes were incubated with the indicated primary antibodies including anti-KIAA0101 (1:2000) antibodies overnight at 4°C. GAPDH (1:1000) was stained as a loading control followed by incubation with horse radish peroxidase-conjugated secondary antibodies. The protein signals were detected an5.9 (h)]TJ0.155 Tw 0 -1.2 Td[(t)6 (o 1)19 5n (t) 5 (i)io12 (DS P)k8cwcy i (o e(er)1 (o 1io)12 (12 (5 (l)3 e) 6 (a) 3(w)

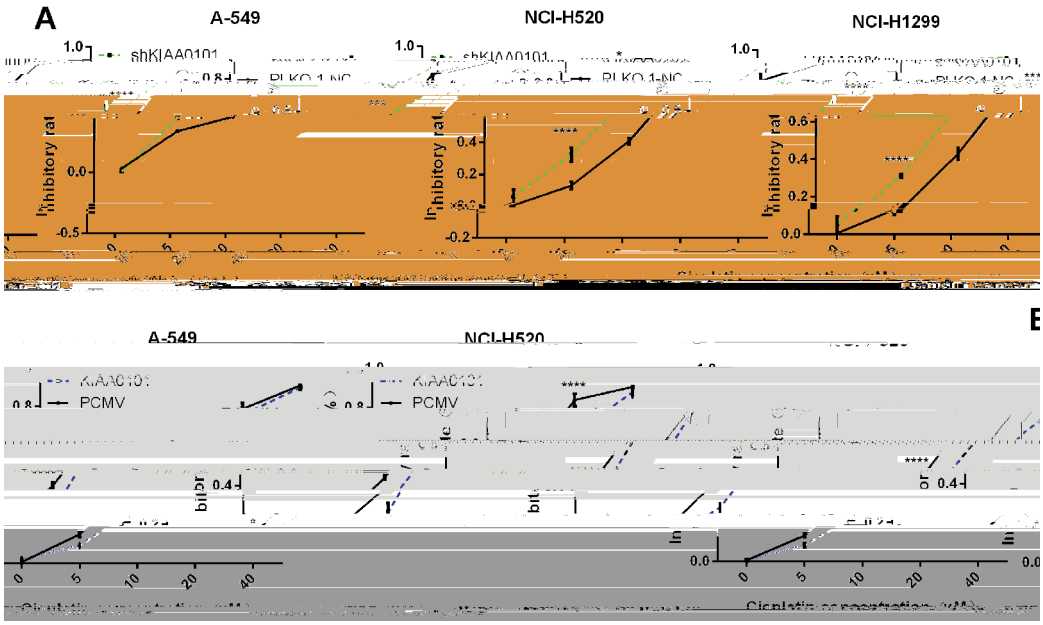

Figure 2: Cell proliferation was determined by MTT assays. (A. Knockout KIAA0101 in A-549, NCI-H520, NCI-H1299 cells, and then treated with different concentrations of cisplatin (0, 5, 10, 20 and 40 μM). The cell viability was detected by MTT and the proliferation inhibition rate was calculated. *P<0.05, ***P<0.001, ****P<0.0001; B. Osareated with dated wsression A-549, NCI-H510, NCI-H1299 cells, and then 6 (MTT)treated with different

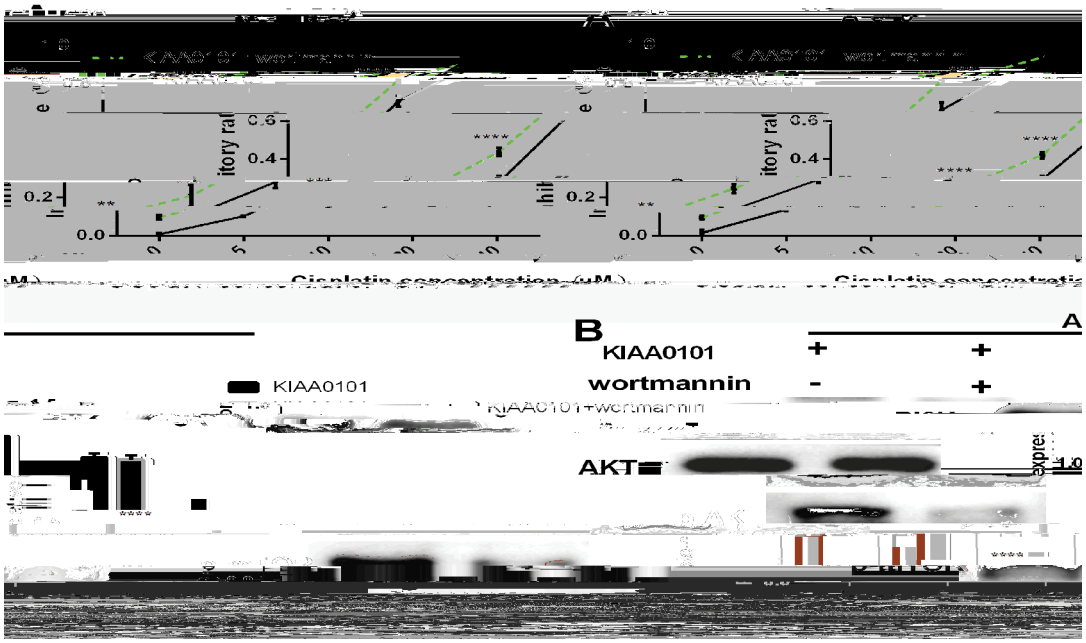

Figure 4: KIAA0101 regulates cisplatin-resistant sensitivity of NSCLC cells by activating PI3K/AKT/mTOR signaling pathway (A. A-549 and NCI-H520 cells are

factor, KIAA0101 does not inhibit DNA replication and cell cycle progression [7,18]. It is closely related to the occurrence and development of cancer, but its mechanism of action in cancer is still unclear [4,19]. We found that the expression of KIAA0101 can increased for the medicine application of cisplatin. Then, we further found that compared with the PCMV group, overexpression of KIAA0101 promoted the proliferation of A-549, NCI-H520, NCI-H1299 cells treated with 0, 5, 10, 20, 40 μM cisplatin. Besides, compared with the PLKO.1-NC group, knockdown of KIAA0101 inhibited the proliferation of A549 and NCI-H520 cells treated with 0, 5, 10, 20, 40 μM cisplatin. It means that KIAA0101 as a carcinogen promotes cisplatin resistance in NSCLC.

The PI3K/AKT pathway is an important signal transduction pathway in cells, and it plays an important biological function in cell proliferation, apoptosis, and metabolic function [20]. Its main members are: PI3K, AKT, mTOR (mammalian targe of rapamycin). PI3K/AKT/mTOR pathway inhibits apoptosis and autophagy after activation, and PI3K/Akt/mTOR signaling pathway plays an important role in the development of NSCLC [21]. PI3K is a heterodimer and is composed of a regulatory p85 subunit and a catalytic p110 subunit. The PI3K pathway is involved in cell survival and growth, and can be activated by extracellular factors. Akt, downstream of PI3K, is also considered to be an important factor in cell survival. mTOR is a key downstream molecule of AKT, once phosphorylated AKT activated mTOR and it regulates multiple target genes leading to increased cell proliferation and survival [22]. Activated mTOR can stimulate the eukaryotic cell to promote E4 and cause cell proliferation. Some research found that the dysregulation of PI3K/AKT/mTOR signaling pathway is closely related to the occurrence of NSCLC and cisplatin-resistance [21,23-25]. Hu et al. revealed that PI3K-Akt pathway may include potential therapeutic target molecules in lung cancer chemotherapeutic resistance [25]. Kim et al. show that downregulation of PI3K/mTOR signaling pathway were

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