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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 explore the regulating mechanism of KIAA0101 regulating cisplatin resistance in NSCLC by WB and the rescue experiment. Our research showed that cisplatin had much stronger inhibitory effects, cisplatin-resistance level decreased, and the expression of PI3K, p-Akt, and p-mTOR significantly decreased in NSCLC cells when KIAA0101 expression was knocked down. On the contrary, overexpression of KIAA0101 significantly weakens the inhibitory effect of cisplatin on NSCLC cells, and cisplatin-resistance level, while the expression of PI3K, p-Akt, and p-mTOR significantly increased. When adding PI3K signaling pathway inhibitor wortmannin on the overexpressing of KIAA0101, the role of KIAA0101 in enhancing lung cancer resistance was reversed, and the expression of PI3K, p-AKT, and p-mTOR protein decreased. Our study revealed for the first time that KIAA0101 as a carcinogen promoting cisplatin resistance in NSCLC, and may be regarded as a new target for clinical treatment of non-small cell lung cancer.

Keywords: KIAA0101; Non-small-cell lung cancer; Cisplatin resistance; PI3K/Akt/mTOR

Introduction

Lung cancer is one of the most common tumors in the world and it has highest mortality rate among all malignant tumors [1], 75% to 80% of them are non-small cell lung cancer. At present, platinum-based chemotherapy drugs, including cisplatin (DDP) and carboplatin, supplemented by other types of chemotherapy drugs, are the most effective treatment for NSCLC. However, cisplatin resistance is an important cause of tumor treatment failure and is associated with poor prognosis. Because of complex interaction of multiple factors, the mechanisms that affect cisplatin resistance are complex and diverse.

KIAA0101 is a proliferating cell nuclear antigen (PCNA) related protein [2,3], and is mainly located in the nucleus. Similar to other PCNA-interacting proteins KIAA0101 was binding motif with other PCNA binding proteins (including cyclin-dependent kinase (Cdk) inhibitor P21). It is worth noting that similar to PCNA, KIAA0101 may have prognostic significance in more human cancers [4]. And KIAA0101 has been proven to be a highly expressed oncogene in many human malignancies, such as gastric cancer [5], esophageal cancer [6], breast cancer [7], renal cell carcinoma [8] and hepatocellular carcinoma [9,10]. Zhang et al. [11] found that higher KIAA0101 was expressed in NSCLC and is closely related to poor prognosis through bioinformatics analysis. Kato et al. [12] also proves that KIAA0101 is closely related to the prognosis of NSCLC. However, the biological function of KIAA0101 is still not clear in NSCLC. The objective of this study was to investigate the effect of KIAA0101 on cisplatin resistance in NSCLC, as well as the possible underlying mechanism.

Materials and Methods

Cell cultures

Lung cancer cell (A-549, NCI-H520, NCI-H1299) were purchased from the American Type Culture Collection. And they were maintained in Roswell Park Memorial Institute (RPMI) 1640 (Gibco, CA, USA) supplemented with 10% foetal bovine serum (FBS) (Sigma-Aldrich, New Jersey, USA) and 100μg/ml penicillin-streptomycin (Genom Bio-pharmaceutical Tech, Hangzhou, China). All cells were placed in 5% CO_2, 37°C.

Cell transfection and grouping

The A-549, NCI-H520 and NCI-H1299 cells were plated in 6-well plates at a density of 4×10^5 cells/well, and were cultured overnight. The cells were transfected with lentivirus carrying short hairpin RNA (shRNA) targeting KIAA0101 (5'-GCTTTGTTGAACAGGCATTTA-3') [13]. Then cells were maintained for 24 h and carried out using a Lipofectamine 2000 liposome transfection kit (Takara, Shiga, Japan) according to the manufacturer's protocol, cell were observed 48 h after transfection. Overexpression and knockdown plasmids and control plasmids were constructed and synthesized by GenePharma Company. The cells were divided into shKIAA0101 group (knockdown of KIAA0101), PLKO.1-NC group (transfected with unrelated sequence), KIAA0101 group (overexpression of KIAA0101), PCMV group (transfected with pCDNA 3.1-LincRNA-ROR), and KIAA0101 (overexpression of KIAA0101) + wortmannin (5 μM, a PI3K inhibitor) group.

MTT assay

After transfection or treatment, A-549, NCI-H520 and NCI-H1299 cells were obtained in the logarithmic growth phase and suspended with cell growth solution. The cell density was adjusted to 1.0×10^4 cells/mL, and cells were evenly spread in 96-well plates with 100 μL in each well. After 24 h, different concentrations of cisplatin (0, 5, 10, 20 and 40 μM; Sigma, USA) were added to the cell culture medium. After cultured for 48 h, cells were incubated with MTT (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma) (5 mg/ml) for 4 h. The produced

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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 experi-mental group/OD value of normal group.

Western blot analysis

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 using the enhanced chemiluminescence method and quantified by Scion Image 4.03 software.

A-549, NCI-H520 and NCI-H1299 cells were seeded in 10 cm plates at 2×10^6 cells and maintained for 24 h. 24 h later, 10 μM cisplatin was cultured for 0, 24, 48, 72 h. KIAA0101 protein expression was detected after cell collection and protein extraction (Same as above). The results of WB were analyzed same as above.

After transfection, A-549 and NCI-H520 cells were collected and protein expression of anti-PI3K (1:500), anti-AKT (1:1000), anti-P-AKT (1:1000), anti-P-mTOR (1:1000) was detected. The results of WB were analyzed same as above. All antibodies were purchased from Abcam (Cambridge, MA).

Statistical analysis

Statistical analysis was performed using the SPSS 21.0. The experiment was repeated three times to calculate mean value and measurement data were presented as mean ± SD. Comparisons among multiple groups were using one-way analyses of variance (ANOVAs) □Two-way ANOVA, Sidak's multiple comparisons test and Tukey's multiple comparisons test. P-value < 0.05 was considered statistically significant.

Results

Cisplatin induces KIAA0101 expression in NSCLC cells

A-549, NCI-H520 and NCI-H1299 cells were treated with 0, 5, 10 and 20 μM cisplatin for 48 hours. The results showed that KIAA0101 protein levels increased with increase of cisplatin concentration from 0 to 20 μM. A-549, NCI-H520 and NCI-H1299 cells were treated with 10 μM cisplatin and cultured for 0 hours, 24 hours, 48 hours, and 72 hours respectively. The results showed that KIAA0101 expression increased with therapy time of cisplatin (Figure 1).

KIAA0101 promotes the resistance of NSCLC cells to cisplatin

NSCLC cells were treated with different concentration of cisplatin (0、5、10、20、40uM). And then we detected cell viability and calculated the proliferation inhibition rate by MTT. When A-549 cell treated with cisplatin of 5, 10 uM, the cell proliferation inhibition rate in shKIAA0101 group increased 2.49 times and 1.67 times compared with the PLKO.1-NC group (Figure 2A, P<0.0001). Conversely, when A549 cells treated with cisplatin of 10 uM, the proliferation inhibition rate in KIAA0101 group was 0.65 times compared with the PCMV group (Figure 2B, P<0.0001). In order to avoid the accidental results of the experiment, we compared the cell proliferation rates of shKIAA0101 group and PLKO.1-NC group in NCI-H520 and NCI-H1299 cells (Figure 2A), and compared the cell proliferation rates of KIAA0101 group and PCMV group in NCI-H520 cell (Figure 2B). We obtained similar results, knockdown of KIAA0101, cisplatin has a stronger inhibitory effect on NSCLC cells, and cisplatin resistance reduced. On the contrary, overexpression of KIAA0101 will weaken the inhibitory effect of cisplatin on NSCLC cells and enhance cisplatin resistance.

KIAA0101 activated PI3K/AKT/mTOR signaling pathway

A-549 cells were divided into PLKO.1-NC group and shKIAA0101 group, and they were treated with 10 μM cisplatin, and we used WB to detected the protein expression of PI3K, AKT, p-AKT, p-mTOR, the results showed that the protein expression of PI3K, p-AKT and p-mTOR was significantly reduced after knocking down KIAA0101 (Figure 3A). On the contrary, the protein expression of PI3K, p-AKT and p-mTOR protein expression was significantly increased after overexpression of KIAA0101 in NCI-H520 (Figure 3A). The results of quantitative analysis of gray values showed statistically significant differences (Figure 3B).

KIAA0101 regulates cisplatin resistance in NSCLC cells by activating PI3K/AKT/mTOR signaling pathway

A-549 and NCI-H520 cells are divided into KIAA0101 group and KIAA0101+wortmannin group. NSCLC cells were treated with cisplatin 0, 5, 10, 20 and 40 μM and we used MTT detect the cell viability and proliferation. We found that when treated with 0,5,10 and 20 μM cisplatin, the cell proliferation inhibition rate of KIAA0101+wortmannin group was 15.05, 2.06, 1.57, 1.15 times higher than that of KIAA0101 group (Figure 4A, P<0.01 or P<0.0001). Similar results were obtained in NCI-H520 cells, it indicating that inhibition of PI3K signaling pathway

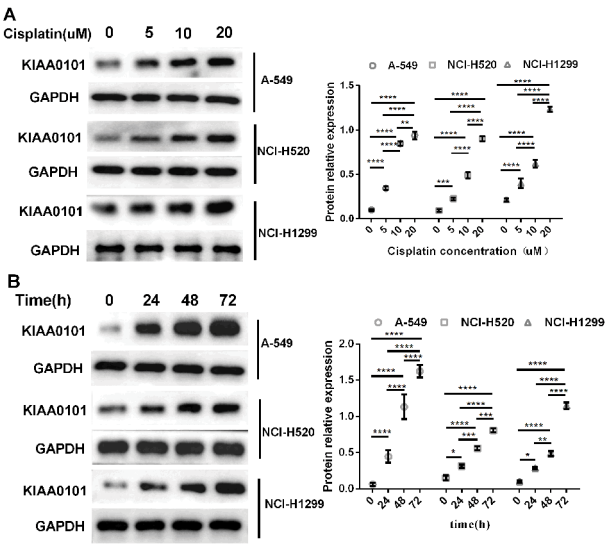

Figure 1: Cisplatin induces the expression of KIAA0101 in NSCLC cells (A. The A-549, NCI-H520, and NCI-H1299 cells were treated with cisplatin at concentrations of 0, 5, 10, and 20 μM for 48 hours, and the KIAA0101 protein expression was detected by WB (**P<0.01,***P<0.001,****P<0.0001); B. Cisplatin at a concentration of 10 μM acted on three types of NSCLC cells A-549, NCI-H520, NCI-H1299 respectively, KIAA0101 protein was detected by WB at 0, 24, 48 and 72 h (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).

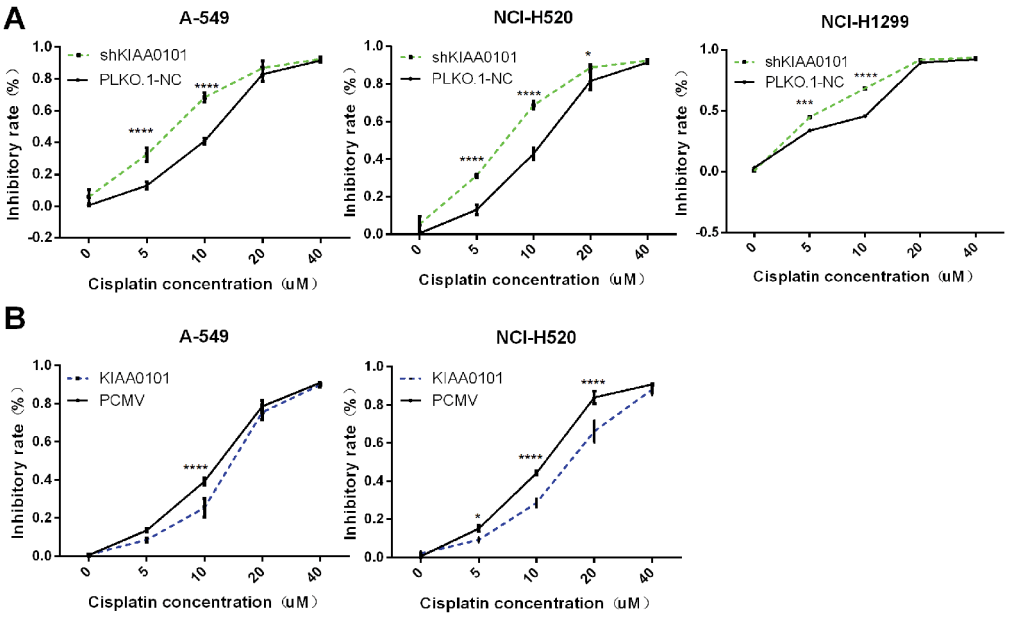

Figure 2: Cell proliferation was determined by MTT assays. (A. Knockout KIAA0101 in A-549, NCI-H520, NCI-H1299 cells, and then treated with different concentrations of cisplatin (0, 5, 10, 20 and 40 μM). The cell viability was detected by MTT and the proliferation inhibition rate was calculated. *P<0.05,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

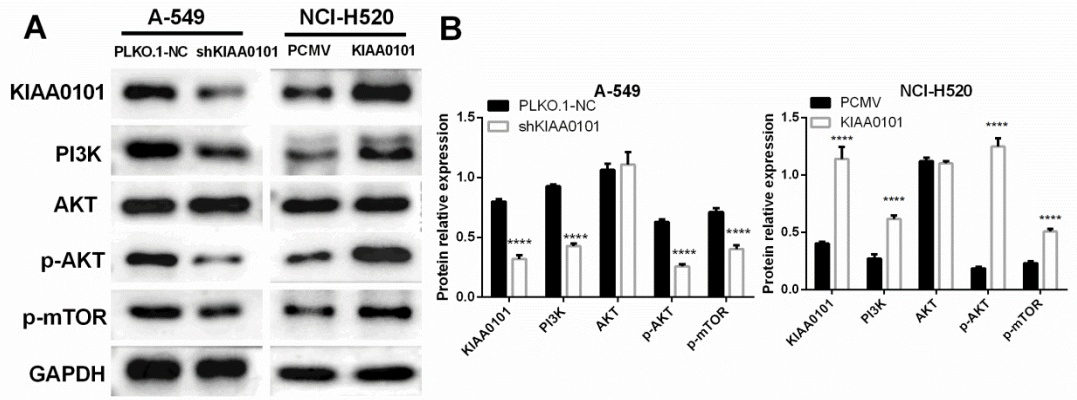

Figure 3: KIAA0101 activates PI3K/AKT/mTOR signaling pathway (A. Detection of PI3K/AKT/mTOR signaling pathway protein expression after knockout and overexpression of KIAA0101 in A-549 and NCI-H520 cells by WB. B. A quantitative analysis of the WB results was performed, ****P<0.0001).

can reverse cisplatin resistance in NSCLC cells by overexpression of KIAA0101. We found that after adding wortmannin, PI3K, p-AKT, p-mTOR protein expression was re-inhibited (Figure 4B).

Discussion

Lung cancer is the most common malignant tumor in the world and the leading cause of cancer-related deaths worldwide. Although the progress has been made in improving the treatment and diagnosis of lung cancer over the past decades, the incidence and mortality rate of lung cancer has not decreased significantly. Non-small cell lung cancer was including: squamous cell carcinoma, adenocarcinoma and large cell lung cancer [14]. For NSCLC patients, its five-year survival rate is only about 18%, because about 65% of NSCLC patients have been diagnosed already at the end stage [14,15]. Although surgical resection is the best treatment option of NSCLC patients, only a small proportion of patients are suitable. Chemotherapy and radiotherapy are widely used for NSCLC therapy, but NSCLC is lowly sensitive to radiotherapy and chemotherapy, it is also an important reason for its high mortality rate ranks first in the global cancer mortality rate [15]. Platinum-based chemotherapy is still the first choice for most advanced NSCLC patients. DDP was based chemotherapy is the common first-line therapy for lung cancer. Its main mechanism were inhibit DNA replication, affect cell transcription and translation, and promote cancer cell apoptosis [16,17]. Cisplatin-acquired resistance were an important factor leading to treatment failure and affecting the survival prognosis of patients.

KIAA0101 is a proliferating cell unclear antigen (PCNA)-associated

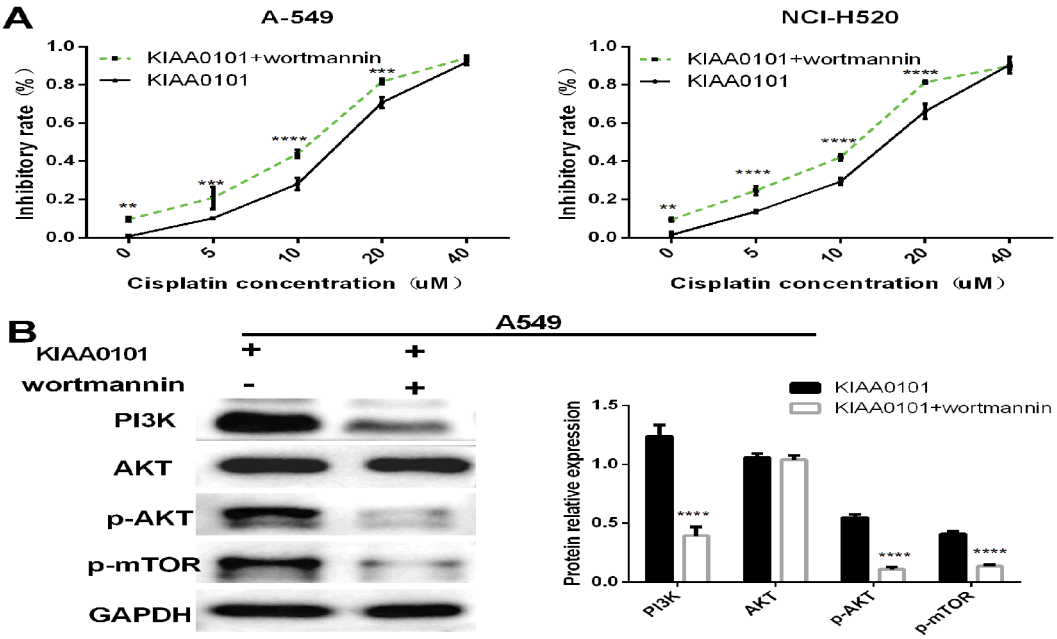

Figure 4: KIAA0101 regulates cisplatin-resistant sensitivity of NSCLC cells by activating PI3K/AKT/mTOR signaling pathway (A. A-549 and NCI-H520 cells are divided into KIAA0101 group and KIAA0101+ wortmannin group, the cells were treated with different concentrations of cisplatin (0, 5, 10, 20, 40 μM),cell viability and calculate proliferation inhibition rate were detected by MTT , compared with KIAA0101 group, **P<0.01, ***P<0.001, ****P<0.0001; B. WB results for the protein expression of PI3K, AKT, p-AKT and p-mTOR of KIAA0101 group and KIAA0101+wortmannin group in A-549 cells, ****P<0.0001).

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 associated with an increase in autophagy [26].

Conclusion

We found that KIAA0101 regulates cisplatin resistance in NSCLC cells by activating PI3K/AKT/mTOR signaling pathway. After adding PI3K signaling pathway inhibitor wortmannin, PI3K, p-AKT, and p-mTOR protein expression were re-inhibited. Although we have demonstrated that KIAA0101 can mediate the resistance of NSCLC to DDP by regulating the PI3K/AKT/mTOR signaling pathway, it is not yet clear how KIAA0101 regulates the PI3K/AKT/mTOR signaling pathway. In future research, we will explore the specific mechanism on NSCLC by KIAA0101 regulates PI3K/AKT/mTOR as well as applying *in vivo*. And it will hopefully provide new possible target for NSCLC diagnosis and therapeutic strategies.

References

1. Pham, N., Bonnen, M. D., & Ghebre, Y. T.
Silent Neoplastic Cardiac Invasion in Small Cell Lung Cancer: A Case Report and Review of the Literature.
Am J Case Rep., 2018; **19**: 619-622.
2. Zhao, H., Chen, M., Wang, J., Cao, G., Chen, W., et al.
PCNA-associated factor KIAA0101 transcriptionally induced by ELK1 controls cell proliferation and apoptosis in nasopharyngeal carcinoma: an integrated bioinformatics and experimental study.
Aging., 2020; **12**(7): 5992-6017.
3. Jin, C., Liu, Z., Li, Y., Bu, H., Wang, Y., et al.
PCNA-associated factor P15(PAF) , targeted by FOXM1, predicts poor prognosis in high-grade serous ovarian cancer patients.
Int J Cancer., 2018; **143**(11): 2973-2984.
4. Yu, P., Huang, B., Shen, M., Lau, C., Chan, E., et al.
p15 PAF, a novel PCNA associated factor with increased expression in tumor tissues. Oncogene., 2001; **20**(4): 484-489.
5. Wang, Z., Dang, C., Yan, R., Zhang, H., Yuan, D., et al.
Screening of cell cycle-related genes regulated by KIAA0101 in gastric cancer.
South Med J., 2018; **38**(10): 1151-1158.

6. Cheng, Y., Li, K., Diao, D., Zhu, K., Shi, L., et al.
Expression of KIAA0101 protein is associated with poor survival of esophageal cancer patients and resistance to cisplatin treatment in vitro.
Lab Invest., 2013; **93**(12): 1276-1287.

7. Lv, W., Su, B., Li, Y., Geng, C., & Chen, N.
KIAA0101 inhibition suppresses cell proliferation and cell cycle progression by promoting the interaction between p53 and Sp1 in breast cancer.
Biochem Biophys Res Commun., 2018; **503**(2): 600-606.

8. Fan, S., Li, X., Tie, L., Pan, Y., & Li, X.
KIAA0101 is associated with human renal cell carcinoma proliferation and migration induced by erythropoietin.
Oncotarget., 2016; **7**(12): 13520-13537.

9. Liu, L., Liu, Y., Chen, X., Wang, M., Zhou, Y., et al.
Variant 2 of KIAA0101, antagonizing its oncogenic variant 1, might be a potential therapeutic strategy in hepatocellular carcinoma.
Oncotarget., 2017; **8**(27): 43990-44003.

10. Zhang, T., Guo, J., Gu, J., Chen, K., Wang, Z., et al.
KIAA0101 is a novel transcriptional target of FoxM1 and is involved in the regulation of hepatocellular carcinoma microvascular invasion by regulating epithelial-mesenchymal transition.
J Cancer., 2019; **10**(15): 3501-3516.

11. Zhang, L., Peng, R., Sun, Y., Wang, J., Chong, X., et al.
Identification of key genes in non-small cell lung cancer by bioinformatics analysis.
Peer J., 2019; **7**(7): e8215.

12. Kato, T., Daigo, Y., Aragaki, M., Ishikawa, K., Sato, M., et al.
Overexpression of KIAA0101 predicts poor prognosis in primary lung cancer patients.
Lung Cancer., 2012; **75**(1): 110-118.

13. Lu, S., & Archer, M. C.
Sp1 coordinately regulates de novo lipogenesis and proliferation in cancer cells.
Int J Cancer., 2010; **126**(2): 416-425.

14. Herbst, R. S., Heymach, J. V., & Lippman, S. M.
Lung Cancer.
N Engl J Med., 2008; **359**(10): 1367-1380.

15. Reck, M., Heigener, D. F., Mok, T., Soria, J. C., & Rabe, K. F.
Management of non-small-cell lung cancer: recent developments.
Lancet., 2013; **382**(9893): 709-719.

16. Fennell, D. A., Summers, Y., Cadranel, J., Benepal, T., Christoph, D. C., et al.
Cisplatin in the modern era: The backbone of first-line chemotherapy for non-small cell lung cancer.
Cancer Treat Rev., 2016; **44**(1): 42-50.

17. Sarin, N., Engel, F., Kalayda, G. V., Mannewitz, M., Cinatl, J., et al.
Cisplatin resistance in non-small cell lung cancer cells is associated with an abrogation of cisplatin-induced G2/M cell cycle arrest.
PLoS One., 2017; **12**(7): e0181081.

18. Emanuele, M. J., Ciccia, A., Elia, A. E., & Elledge, S. J.
Proliferating cell nuclear antigen (PCNA)-associated KIAA0101/PAF15 protein is a cell cycle-regulated anaphase-promoting complex/cyclosome substrate.
Proc Natl Acad Sci., 2011; **108**(24): 9845-9850.

19. Petroziello, J., Yamane, A., Westendorf, L., Thompson, M., McDonagh, C., et al.
Suppression subtractive hybridization and expression profiling identifies a unique set of genes overexpressed in non-small-cell lung cancer.
Oncogene., 2004; **23**(46): 7734-7745.

20. Wang, S. S., Chen, Y. H., & Chen, N.
Hydrogen sulfide promotes autophagy of hepatocellular carcinoma cells through the PI3K/Akt/mTOR signaling pathway.
Cell Death Dis 2017; **8**(3): 2688.

21. Shi, H., Pu, J., Zhou, X. L., Ning, Y. Y., & Bai, C.
Silencing long non-coding RNA ROR improves sensitivity of non-small-cell lung cancer to cisplatin resistance by inhibiting PI3K/Akt/mTOR signaling pathway.
Tumour Biol J Int Soc Oncodevelop Biol Med., 2017; **39**(5): 568.

22. Fumarola, C., Bonelli, M. A., Petronini, P. G., & Alfieri, R. R.
Targeting PI3K/AKT/mTOR pathway in non small cell lung cancer.
Biochem Pharmacol., 2014; **90**(3): 197-207.

23. Teng, X., Fan, X. F., & Li, Q.
XPC inhibition rescues cisplatin resistance via the Akt/mTOR signaling pathway in A549/DDP lung adenocarcinoma cells.
Oncol Rep., 2019; **41**(3): 1875-1882.

24. Xia, A., Li, H., Li, R., Lu, L., & Wu, X.
Co-treatment with BEZ235 enhances chemosensitivity of A549/DDP cells to cisplatin via inhibition of PI3K/Akt/mTOR signaling and downregulation of ERCC1 expression.
Oncol Rep., 2018; **40**(4): 2353-2362.

25. Hu, C. F., Huang, Y. Y., Wang, Y. J., & Gao, F. G.
Upregulation of ABCG2 via the PI3K-Akt pathway contributes to acidic microenvironment-induced cisplatin resistance in A549 and LTEP-a-2 lung cancer cells.
Oncol Rep., 2016; **36**(1): 455-461.

26. Kim, K. W., Myers, C. J., Jung, D. K., & Lu, B.
NVP-BEZ-235 enhances radiosensitization via blockade of the PI3K/mTOR pathway in cisplatin-resistant non-small cell lung carcinoma.
Genes Cancer., 2014; **5**(7-8): 293-302.