European Review for Medical and Pharmacological Sciences 2019; 23: 5229-5234

Long noncoding LUCAT1 promotes cisplatin resistance of non-small cell lung cancer by promoting IGF-2

W. WANG[1], M.-L. DONG[2], W. ZHANG[3], T. LIU[4]

[1]Department of Pharmacy, Yantai Yuhuangding Hospital, Yantai, China
[2]Sterile Supply Room, Yantai Yuhuangding Hospital, Yantai, China
[3]Department of Thoracic Surgery, Yantai Yuhuangding Hospital, Yan[illegible]
[4]Department of Thoracic Surgery, The Second People's Hospital of [illegible]ang, [illegible]

Wei Wang, Meili Dong and Wei Zhang contributed equally to t[illegible]rk

Abstract. – OBJECTIVE: Drug-resistance remains a huge problem in the therapy of malignant tumors including non-small cell lung cancer (NSCLC). Several researches have proved that long noncoding RNAs (lncRNAs) contributes to drug-resistance in NSCLC. LncRNA LUCAT1 was explored to identify how it functions in the cisplatin-resistance of NSCLC patie[illegible]

MATERIALS AND METHODS: Real Tim[illegible]titative Polymerase Chain Reaction (RT[illegible]R) was utilized to detect LUCAT1 expressi[illegible]n A549/DDP cells and A549 cells. Then, we [illegible]ducted cell counting kit-8 (CCK-8) assay a[illegible] flow cytometric analysis to det[illegible]e functio[illegible] of LUCAT1 on the resistance[illegible] cells to[illegible] cisplatin. Furthermore, the [illegible]ntial [illegible]hanism was explored by mechani[illegible]ssays.

RESULTS: LUCAT1 e[illegible]sion [illegible] cells was higher tha[illegible] pa[illegible] cells. [illegible] sides, cell apoptos[illegible]as i[illegible], cell cycle distribution was c[illegible]ged, and r[illegible]ce to cisplatin was prom[illegible]after LUCAT[illegible]overexpressed in A5[illegible]rthermore, t[illegible]overexpression of L[illegible]AT1 c[illegible]upregulate the IGF-2 expressio[illegible]A549/DDP.

CONC[illegible]ONS: We sugg[illegible]t LUCAT1 regulates [illegible]cycle, cell apoptos[illegible]of NSCLC cells and t[illegible]esista[illegible]to cisplatin through targeting[illegible]2 and[illegible]d be a possible target for NSCLC[illegible]

Key Words:
[illegible]NA, LC[illegible]CLC, IGF-2, Cisplatin.

Introduction

[illegible]g cancer is one of the most frequent malig[illegible]es in the world which is also the leading cause of tumor-related deaths globally, accounting for almost 1.3 million deaths annually[1]. Non-small cell lung cancer (NSCLC), one prominent type of lung [illegible]er, represents the majority of cancer diag[illegible]ed. NSCLC patients at early [illegible] were t[illegible]d traditionally with surgery. How[illegible] NSCLC cases are unfortunately diagnosed at an advanced stage without the opportunity to take curable surgery which contributes to the poor survival rate. Platinum-based [illegible]herapeutics is the main intervention for advanced cases, such as cisplatin or carboplatin. However, continuous and/or multiple administrations often result in the development of drug resistance leading to the failure of therapeutic management.

Cisplatin covalently activates the DNA-damage response, induces cell apoptosis and cell cycle arrest. Accumulating evidence has indicated that drug-resistance is closely modified by long noncoding RNAs (lncRNAs) which is longer than 200 nt without the ability of coding protein. For example, through increasing the stability of nuclear SREBP-1c protein, lncRNA MALAT1 enhances hepatic steatosis and insulin resistance[2]. By regulating the PTEN-PI3K/Akt signal pathway, lncARSR facilitates doxorubicin resistance of hepatocellular carcinoma which may serve as a potential therapeutic target and prognostic biomarker[3]. Overexpression of lncRNA SRLR decreases the responses to intrinsic sorafenib therapy in renal cell carcinoma[4]. Through sequestration of miR-23b-3p, lncRNA MALAT1 promotes chemo-induced autophagy in gastric cancer[5]. Moreover, lncRNA ANRIL promotes the progression of lung adenocarcinoma and contributes to paclitaxel resistance[6]. However, the

Corresponding Author: Tao Liu, BM; e-mail: liutao567@163.com

clinical role of LUCAT1 in cisplatin-resistance remains unknown. Therefore, we conducted this study and found that LUCAT1 could regulate cell apoptosis, cell cycle and cisplatin resistance in NSCLC *via* upregulating IGF-2.

Materials and Methods

NSCLC Cell Lines and Cell Transfection

A549/DDP cells and A549 cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Invitrogen, Carlsbad, CA, USA) added with 10% fetal bovine serum (FBS, Gibco, Rockville, MD, USA). Besides, the incubator for cell culture was consist of 5% CO_2 at 37°C. Lentivirus targeting LUCAT1 (LUCAT1/shRNA; GenePharma, Shanghai, China) was cloned into pLenti-EF1a-EGFP-F2A-Puro-CMV, which were then transfected in NSCLC cells.

RNA Extraction and Real Time-Quantitative Polymerase Chain Reaction (RT-qPCR)

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was utilized for extracting total RNA, which was then reversely transcribed to complemental deoxyribose nucleic acid (cDNA) through reverse Transcription Kit (TaKaRa Biotechnology Co. Ltd., Dalian, China). Following were the primers used for RTqPCR: LUCAT1, forward: 5'-CTATC-CCTTTCTCTAAGAA-3' and reverse: 5'-CTTCT-GCAAAAACGTGCTG-3'; and for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) 5'-CCACATCGCTCA-CAC-3' and reverse: 5'-ACCAGGCGCC-TACG-3'. Normal cycle was as follows: 95°C for 30 sec, 5 sec for 40 cycles at 95°C, 60°C for

Cell Counting Kit-8 (CCK-8) Assay

Half maximal inhibitory concentration (IC_{50}) of cisplatin, an important factor of cisplatin resistance, was detected by CCK-8 assay (Dojindo, Kumamoto, Japan). Different concentrations of cisplatin were used to treat the cells. Absorbance was measured at 450 nm.

Flow Cytometry Analysis

For apoptosis assay, flow cytometry binding buffer (500 µL) was added after harvested cells were washed twice using ice-cold. A mixture containing 5 µL Annexin V/FICC (fluorescein isothiocyanate) and 5 µL Propidium Iodide (PI; BD, Franklin Lakes, NJ, USA) was used for staining these cells for 15 min in the dark. Then, they were added with 400 microliters binding buffer. FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) was performed to analyze cell apoptosis. For cell cycle assay, 2×10^5/mL cells were diluted by PBA in 75% ice-cold ethanol overnight. And the cells were stained with propidium iodide (PI; 50 µL BD Bioscience, Franklin Lakes, NJ, USA) in dark for 30 min at 4°C. Then, the were measured with a flow cytometer (FACSCalibur BD Bioscience, Franklin Lakes, NJ, USA).

Western Blotting Analysis

Cell lysis containing NaCl and Triton X-100 was used for extracting total protein. The protein was transferred to nitrocellulose membrane by Sodium Dodecyl Sulphate (SDS) polyacrylamide gel. Then, 5% non-fat milk was used for blocking membranes within Tris-Buffered Saline and Tween (TBST). Specific primary antibody was then utilized for incubating the membrane at 4°C overnight. Next, the samples were incubated with second antibody at room temperature for 30 min. Protein bands were visualized by ImageQuant LAS 4000 (Pittsburgh, USA) with enhanced chemiluminescence (ECL) reagents (Pierce, Rockford, IL, USA).

Statistical Analysis

Statistical analysis was conducted by Statistical Product and Service Solutions (SPSS) 20.0 (SPSS, Chicago, IL, USA). The Student's *t*-test was used to detect the related changes by the method of $2^{-\Delta\Delta CT}$. $p<0.05$ was considered statistically significant.

Results

The Expression of LUCAT1 in Cisplatin-Resistance NSCLC Cells

The IC_{50} of cisplatin in A549/DDP cells was higher than that in A549 cells (Figure 1A). LUCAT1 was downregulated in A549 cells compared with A549/DDP cells (Figure 1B).

Treating With Cisplatin in A549 Cells Upregulated LUCAT1 Expression

The A549 cells were treated with different concentrations of cisplatin. RT-qPCR results showed that the LUCAT1 expression of these treated cells was increased in accordance with the elevated concentrations of cisplatin (Figure 2).

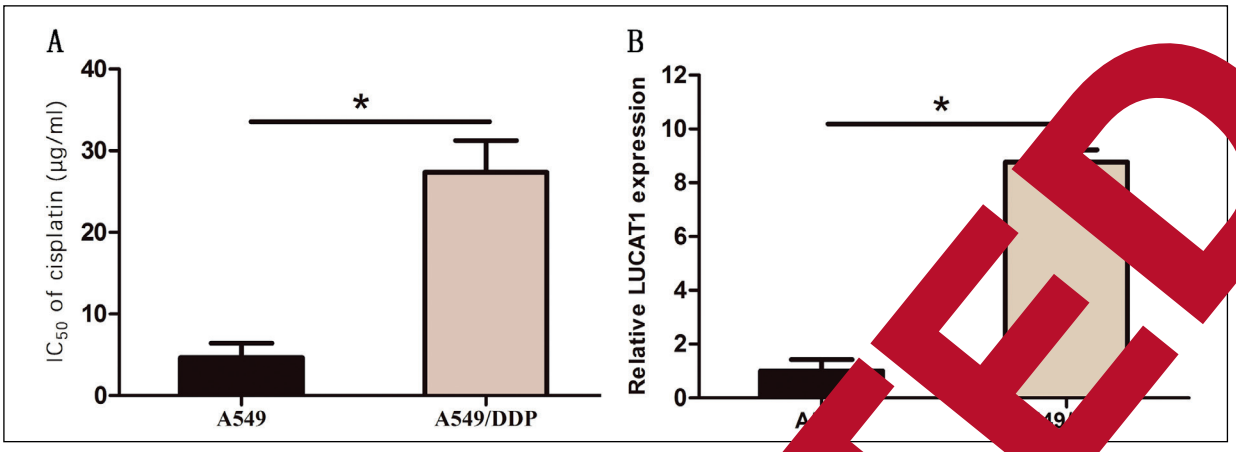
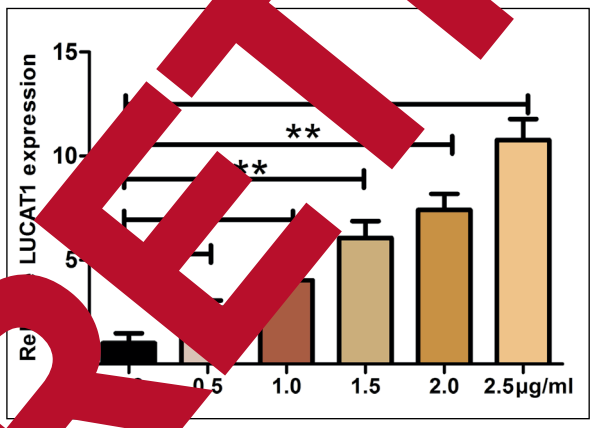


Figure 1. Expression levels of LUCAT1 were increased in A549/DDP cells ... of cisplatin wa... higher in A549/DDP cells, as compared with that of the A549 cells. ***B,*** Expression levels of ...AT1 re... GAPDH were determined in the A549/DDP and A549 cells by RT-qPCR. GAPDH was used as an internal control. *p<0...

Cisplatin Resistance Was Promoted by Overexpression of LUCAT1 in A549 Cell Line

RT-qPCR results also showed that LUCAT1 was significantly upregulated in A549 cells transfected with LUCAT1 lentivirus (LUCAT1) (... re 3A). CCK-8 assay revealed that IC_{50} of ... was increased by upregulating LUCAT1 in ...49 cells (Figure 3B). Besides, cell apoptosis was ... pressed in the LUCAT1/shRNA group after th... cells were treated with different c... ntrations ... cisplatin (Figure 4A). Moreov... subG0/G1 phases in the LUCAT1/s... A gro... was decreased after these cells w... eated w... ifferent concentrations of cisplatin ... re 4...



...ure 2. LUCAT1 was upregulated in A549 cells treated ...th cisplatin. A549 cells were cultured in various con... ...s of cisplatin (0.0, 0.5, 1.0, 1.5, 2.0 or 2.5 μg/mL) for 2... LUCAT1 expression was evaluated by RT-qPCR. GAPDH was used as an internal control. *p<0.05.

...erexpression of LUCAT1 Enhanced ...platin Resi...nce in A549/DDP Cells ...ough Pron...ing IGF-2

...performe... RT-qPCR in A549/DDP cells trans... LUCAT1 lentivirus. Results revealed that IGF-2 expression was higher in the LUCAT1 lentivirus group compared with that in ... group (Figure 4C). Western blot analysis ...sults ...vealed that IGF-2 of A549/DDP cells was higher-expressed in the LUCAT1 lentivirus group compared with that in the control group (Figure 4D).

Discussion

The resistance to chemotherapy drugs remains a vital factor of prognosis for patients with lung cancers and brings a huge burden to patients and society. Researches have revealed that ncRNAs play an important role in the regulation of drug-resistance in NSCLC. For instance, by targeting alpha1,2-fucosyltransferase-1, downregulation of miR-339-5p induces Taxol resistance in small-cell lung cancer[7]. LncRNA UCA1 contributes to non-T790M acquired resistance to EGFR-TKIs in EGFR-mutant NSCLC by activating the AKT/mTOR pathway[8]. LncRNA TUG1 promotes cell proliferation, cell migration, and chemoresistance in small cell lung cancer cells through the regulation of LIMK2b *via* EZH2[9]. The silence of lncRNA AK001796 inhibits cellular cisplatin resistance and cell viability in NSCLC[10].

Some studies have indicated that LUCAT1 acts as an oncogene and promotes cell proliferation, metastasis, and drug-resistance in many tumors.

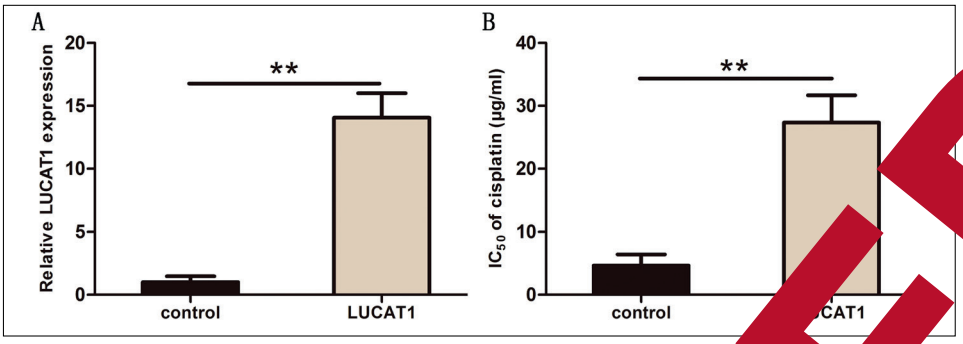
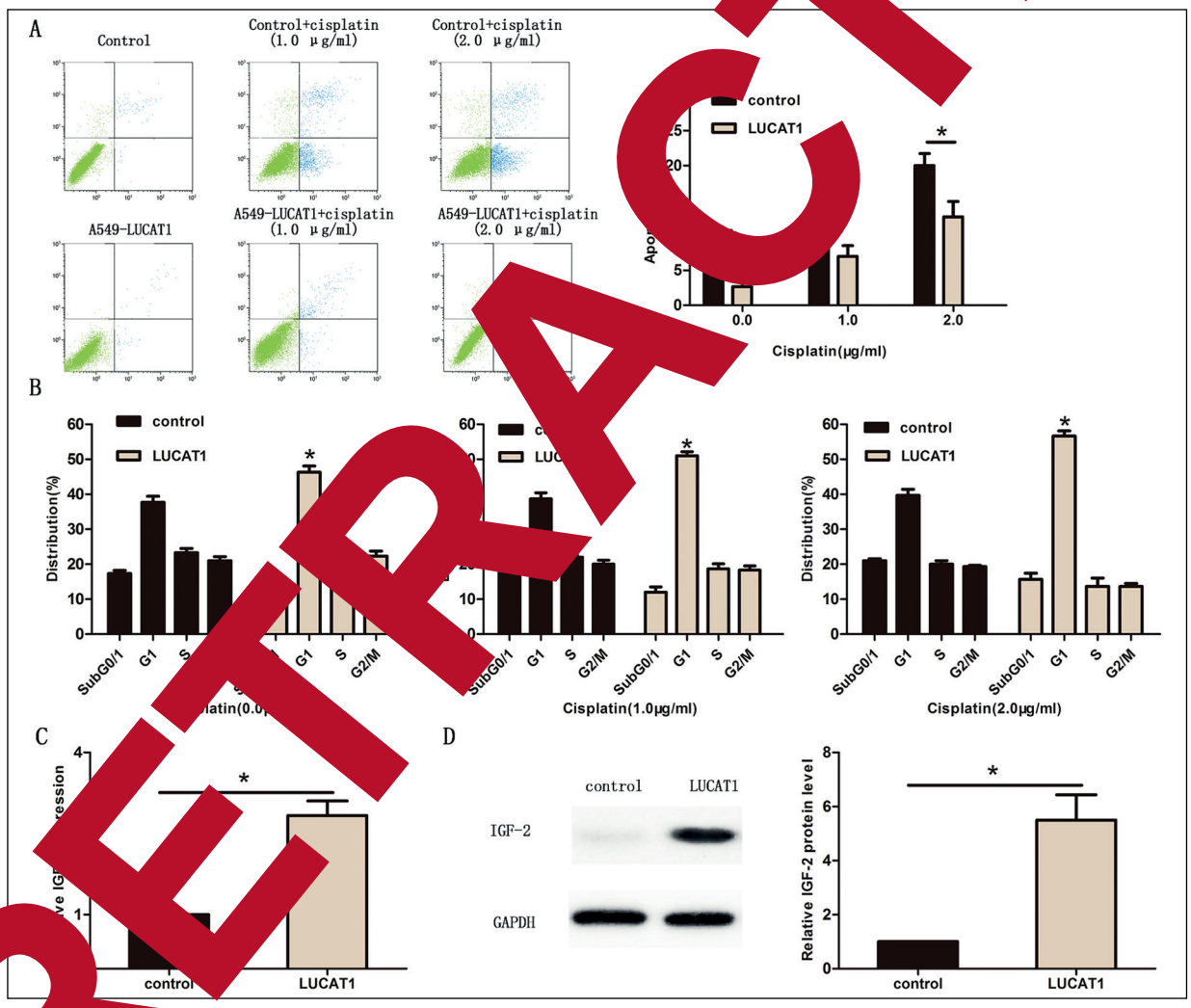


Figure 3. Overexpression of LUCAT1 increased IC50 values of cisplatin in A549 cells. [illegible]er A549 [illegible]s were transfected with LUCAT1 lentivirus, the overexpression efficiency was detected by RT-qPCR. GA[illegible] was used as an internal control. ***B,*** IC_{50} values of cisplatin in A549 cells transfected with control and LUCAT1 lentivirus [illegible] by CCK-8 assay. The results represent the average of three independent experiments. *p<0.05.



Fi[illegible] [illegible]ression of LUCAT1 promoted the resistance of A549/DDP to cisplatin by upregulating IGF2. ***A,*** Cell apoptosis [illegible]ed by [illegible]nt concentrations (0.0, 1.0 or 2.0 μg/mL) of cisplatin was detected in A549 cells transfected with control and [illegible]AT1 lentivirus by flow cytometric analysis. ***B,*** Cell cycle distribution induced by different concentrations (0.0, 1.0 or 2.0 [illegible]L) of cisplatin was detected in A549 cells transfected with control and LUCAT1 lentivirus by flow cytometric analysis. ***C,*** [illegible]A expression levels of IGF-2 in A549/DDP cells transfected with control and LUCAT1 lentivirus were analyzed by RT-qPCR. ***D,*** Protein expression levels of IGF-2 in A549/DDP cells transfected with control and LUCAT1 lentivirus were analyzed by Western blot assay. GAPDH was used as a control. The results represent the average of three independent experiments. *p<0.05.

LUCAT1 (lung cancer associated transcript 1) was firstly found in the airway epithelium of cigarette smokers[11]. LUCAT1 promotes malignancy and progression of ovarian cancer through regulating miR-612/HOXA13 pathway[12]. Knockdown of LUCAT1 depresses the cell ability of viability and invasion in glioma through modulating the expression level of miR-375[13]. Through modulating miR-200c/ABCB1 axis, the overexpression of LUCAT1 promotes cell proliferation and cell invasion in osteosarcoma and contributes to methotrexate resistance[14]. In this study, LUCAT1 was upregulated in A549/DDP cells compared with A549 cells. Besides, the LUCAT1 was upregulated after the dose of cisplatin for treating A549 cells increased. In addition, cisplatin-induced apoptosis of A549 cells was inhibited *via* overexpression of LUCAT1. The percent of A549/DDP cells at subG0/G1 phases was decreased in the LUCAT1 lentivirus group after treating with different doses of cisplatin.

Inhibiting apoptosis and mitosis contributes to the modulation of drug-resistance. IGF2 (insulin-like growth factor 2), as a novel oncogene in tumors, has been reported to particip in the regulation of cell apoptosis and mite example, the overexpression of IGF2 is r k-ably correlated with the sensitivity of colo l cancer tumor to the IGF1R/INSR inhibitor 885578[15]. Induced by Id1, autocri endocri of IGF-II promotes the progre phagea cancer and suppresses the re se to mother-apy[16]. Through regulatin e IGF2 CD44-IGF2 pathway, CD44(+) bla cell proliferation and ug e in breast cancer[17]. Our work wed tha mRNA expression of IGF-2 upregulated 49/DDP cells *via* overe f LUCAT1, d IGF-2 protein was u gulate e overexpression of LUCAT1 i 549/DDP ce

Conclusions

We d rated LUCAT1 could inhibit popto g cell cycle, and promote the latin-r ce of NSCLC cells *via* upregu g IGF-2, which suggested that LUCAT1 ca al target for improve drug-resis- in NS C patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Jun T, Zheng FS, Ren KM, Zhang HY, JZ. Long non-coding RNA UCA gulates t proliferation, migration and in n of human lung cancer cells by modula expression of microRNA-143. Eur Rev Me acol Sci 2018; 22: 8343-8352.
2) Yan C, Chen J, Chen ng noncod MALAT1 promotes h c steatosis and resistance by incre g nucle REBP-1c p-tein stability. Sci 016; 6 40.
3) Li Y, Ye Y, Feng Q coding ncar-sr promotes rubic tance i patocel-lular carci via mod -PI3K/Akt pathway Biochem 20 498-4507.
4) Xu Z, D, Liu B, Chen , Bao Y, Wu Z, Wu n H, L g J, Liu J, Sun S, Qu L, Wang L. Long noncodi A-SRLR elicits intrinsic b resistance oking IL-6/STAT3 ax-enal cell carcino Oncogene 2017; 36: 1965-1977.
5) YiRen H, Ying Y, Sunwu Y, Keqin L, Xiaochun T, Senrui C, End XiZhou L, Yanfan C. Long non-oding RNA AT1 regulates autophagy asso-d chem istance via miR-23b-3p seques-ic cancer. Mol Cancer 2017; 16: 174.
6) Xu R, Mao Y, Chen K, He W, Shi W, Han Y. The long coding RNA ANRIL acts as an oncogene and butes to paclitaxel resistance of lung adenocarcinoma A549 cells. Oncotarget 2017; 8: 39177-39184.
7) Gan CZ, Li G, Luo QS, Li HM. miR-339-5p down-regulation contributes to Taxol resistance in small-cell lung cancer by targeting alpha1,2-fucosyltransferase 1. IUBMB Life 2017; 69: 841-849.
8) Cheng N, Cai W, Ren S, Li X, Wang Q, Pan H, Zhao M, Li J, Zhang Y, Zhao C, Chen X, Fei K, Zhou C, Hirsch FR. Long non-coding RNA UCA1 induces non-T790M acquired resistance to EGFR-TKIs by activating the AKT/mTOR pathway in EGFR-mutant non-small cell lung cancer. Oncotarget 2015; 6: 23582-23593.
9) Niu Y, Ma F, Huang W, Fang S, Li M, Wei T, Guo L. Long non-coding RNA TUG1 is involved in cell growth and chemoresistance of small cell lung cancer by regulating LIMK2b via EZH2. Mol Cancer 2017; 16: 5.
10) Liu B, Pan CF, Ma T, Wang J, Yao GL, Wei K, Chen YJ. Long noncoding RNA AK001796 contributes to cisplatin resistance of nonsmall cell lung cancer. Mol Med Rep 2017; 16: 4107-4112.
11) Thai P, Statt S, Chen CH, Liang E, Campbell C, Wu R. Characterization of a novel long noncoding RNA, SCAL1, induced by cigarette smoke and elevated in lung cancer cell lines. Am J Respir Cell Mol Biol 2013; 49: 204-211.
12) Yu H, Xu Y, Zhang D, Liu G. Long noncoding RNA LUCAT1 promotes malignancy of ovarian cancer

through regulation of miR-612/HOXA13 pathway. Biochem Biophys Res Commun 2018; 503: 2095-2100.

13) Gao YS, Liu XZ, Zhang YG, Liu XJ, Li LZ. Knockdown of long noncoding RNA LUCAT1 inhibits cell viability and invasion by regulating miR-375 in glioma. Oncol Res 2018; 26: 307-313.

14) Han Z, Shi L. Long non-coding RNA LUCAT1 modulates methotrexate resistance in osteosarcoma via miR-200c/ABCB1 axis. Biochem Biophys Res Commun 2018; 495: 947-953.

15) Sanderson MP, Hofmann MH, Garin-Chesa P, Schweifer N, Wernitznig A, Fischer S, Jeschko A, Meyer R, Moll J, Pecina T, Arnhof H, Weyer-Czernilofsky U, Zahn SK, Adolf GR, Kraut N. The IGF1R/INSR inhibitor BI 885578 selectively inhibits growth of IGF2-overexpressing colorectal cancer tumors and potentiates the efficacy of anti-VE
Mol Cancer Ther 2017; 16: 2223-22

16) Li B, Tsao SW, Chan KW, Ludwig D ovosyadlyy R, Li YY, He QY, Cheung AL. Id1-i d IGF-II and its autocrine/endocrine promot cophageal cancer progression and c emores -impli-cations for IGF-II and R-target apy
Clin Cancer Res 2014 2651-2662.

17) Liu Y, Yu C, Wu Y X, Su ou C, Xi
CD44(+) fibroblas crease ast cancer cell survival and drug IGF2B D44-IGF2 signalli Ce ed 201 1979-1988.