European Review for Medical and Pharmacological Sciences 2019; 23: 1102-1107

Long noncoding RNA AK027294 acts as an oncogene in non-small cell lung cancer by up-regulating STAT3

B. CHEN[1,2], C.-H. LING[1]

[1]Department of Respiratory Medicine, The First Affiliated Hospital of Soochow University, Suzhou, China
[2]Department of Oncology, Shanghai Pulmonary Hospital, Shanghai Tongji University, Shanghai, China

Abstract. – OBJECTIVE: Recent researches have proved that long noncoding RNAs (lncRNAs) play an important role in multiple diseases, including malignant tumors. The aim of this study was to explore the exact role of lncRNA AK027294 in the development of non-small cell lung cancer (NSCLC), and to investigate the possible underlying mechanism.

PATIENTS AND METHODS: Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was utilized to detect the AK027294 expression in NSCLC patients. Then, cell counting kit-8 (CCK-8) assay, colony formation assay, and 5-Ethynyl-2'-deoxyuridine (EdU) incorporation assay were performed, respectively. Furthermore, RT-qP Western blot assay were used to explo potential mechanism.

RESULTS: The expression level of AK02 NSCLC samples was significantly higher than t of adjacent tissues. Subsequent f nal assay showed that the growth abili LC cells was markedly inhibited after 27294 ence. In addition, after AK027294 k -down expression of signal transduce activ scription 3 (STAT3) w re down-regulated. Furthermore, esults strated that the STAT3 expres was posit orrelated with the AK0272 ssion in NS ssues.

CONCLUSI ove results ndicated that AK02729 ould e the growth ability of NSCLC up-regulating T3. Our findings suggest hat AK027294 m be a potential theran c targ for NSCLC.

Key
Lo ng RN K027294, Non-small cell lung can CLC), S 3.

Introduction

Lung cancer is one of the leading causes of can- lated death globally. Meanwhile, it remains a pu reat to the society[1]. In 2016, approximately 224,390 cases of lung cancer were diagnosed in the US, including n and fe atient China, the total num of newly di ng cancer patients wa in 2011[3]. N all cell lung cancer (N LC) nts for 85% of lung cancer cases[4]. A great adva s been achieved in explori molecular tu nesis and therapeuti ment. However, the -year survival rate of LC patients remains lower than 15%[5]. There- , it is an urg need to realize the underlying cular mecha m of NSCLC and to search n rapeutic ets for NSCLC patients.

ng RNAs (lncRNAs) are a type of non-pr coding RNAs with longer than 200 cleotides in length. Numerous studies have shown NAs are a new frontier in the research of ang t diseases. For instance, the up-regulation of PVT1 promotes the proliferation, cell cycle progression and metastasis of melanoma cells[6]. Through modulating the signal transducers and activators of transcription 1-mitogen-activated protein kinase (STAT1-MAPK) signal pathway, down-regulation of lncRNA P7 facilitates the proliferation of hepatocellular carcinoma cells. Meanwhile, lncRNA P7 is associated with unfavorable prognosis[7]. By sponging miR-124-3p, lncRNA OGFRP1 has been reported to participate in the proliferation of NSCLC cells[8]. In addition, lncRNA AF147447 represses the proliferation and invasion of gastric cancer infected with *Helicobacter pylori* through regulating miR-34c expression and targeting MUC2[9]. However, the exact function of lncRNA AK027294 in NSCLC, as well as the potential molecular mechanism, have not been elucidated so far.

Patients and Methods

Tissue Samples

A total of 50 paired NSCLC tissues and adjacent tissues were obtained from NSCLC patients who underwent surgery at Shanghai Pulmonary Hospital. No radiotherapy or chemotherapy was

Corresponding Author: Chunhua Ling, MD, Ph.D; e-mail: linchunhua88@hotmail.com

performed before surgery. All fresh tissues collected from the surgery were stored immediately at –80°C for subsequent use. This study was approved by the Review Board of Shanghai Pulmonary Hospital.

Cell Culture

Human NSCLC cell lines (A549, SPCA1, and H358) and normal human bronchial epithelial cell line (16HBE) were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Rockville, MD, USA) consisting of 10% fetal bovine serum (FBS; Invitrogen Life Technologies, Carlsbad, USA) and penicillin. Besides, all cells were maintained in a 5% CO_2, 37°C incubator.

Cell Transfection

The cDNA oligonucleotides specifically targeting AK027294 (sh-AK027294) was synthesized by GenePharma (Shanghai, China), and was inserted into shRNA expression vector pGPH1/Neo. Subsequently, sh-AK027294 was transfected into NSCLC cells according to the instru[illegible] 48 h later, Real Time-quantitative Pol[illegible] Chain Reaction (RT-qPCR) was used to ve[illegible]he transfection efficiency.

Cell Counting Kit-8 (CCK-8) A[illegible]

24, 48, and 72 hours afte[illegible]sfection, the growth ability of cells i[illegible]-well[illegible]tes was assessed following the in[illegible]tions o[illegible]CK8 assay (Dojindo Laborator[illegible]uma[illegible]), respectively. Absorba[illegible] at [illegible] was detected by a spectroph[illegible]eter (T[illegible] Scientific, Waltham, MA, U[illegible].

Colony Fo[illegible]tion A[illegible]

H358 c[illegible] were first se[illegible] into 6-well plate, followed [illegible] culture for 10 day[illegible]en, the formed coloni[illegible]ere fi[illegible] with 10% formaldehyde for 30 [illegible]nd st[illegible] with 0.5% crystal violet for 5 mi[illegible]g[illegible]o Plus [illegible] (Silver Springs, MD, USA) w[illegible] for d[illegible]alysis.

5-[illegible]ynyl-2[illegible]yuridine (EdU) [illegible]rporation Assay

[illegible] the manufacturer's instructions, [illegible] Kit ([illegible]e, Mannheim, Germany) was uti[illegible]d to monitor the proliferation of transfect[illegible]lls. Zeiss Axiophot Photomicroscope (Carl Ze[illegible]berkochen, Germany) was used to capture r[illegible]presentative images.

RNA Extraction and RT-qPCR

TRIzol reagent (Invitrogen, Carlsbad, C[illegible]) was utilized to extract the total RNA i[illegible] cells. The extracted total RNA wa[illegible]erse tra[illegible]scribed to complementary deoxyri[illegible]nucleic acids (cDNAs) through reverse Transc[illegible]Kit (TaKaRa Biotechnology Co., Ltd., D[illegible]lian, C[illegible]Primers used in this study were as f[illegible]s: AK02[illegible]im[illegible]ers forward: 5′-ATGA[illegible]CTATTGGAC[illegible] reverse: 5′-TAAGCAC[illegible]CTGAC[illegible]AT-3′; G[illegible]eraldehyde 3-phosp[illegible]ehyd[illegible]ase (G[illegible]PDH) primers forward: 5′-[illegible]CAGA[illegible]GGG-CAATGCTGG[illegible]d reve[illegible]TGA[illegible]CATG-GACTGTGG[illegible]TTCA-3′. T[illegible]le was as follows: 30 [illegible]°C, 5 sec for a[illegible] of 40 cycles at 95°C, [illegible]5 s[illegible]0°C.

Stati[illegible] Analysis

[illegible]ical Product a[illegible] Service Solutions ([illegible]SS) 17.0 (SPSS[illegible] Chicago, IL, USA) was used [illegible]all statistical[illegible]lyses. The Student *t*-test was [illegible]ormed to co[illegible]e the difference between the t[illegible]ups. Ex[illegible]mental data were presented as mea[illegible]ard deviation). $p<0.05$ was considered [illegible]ically significant.

Results

Expression Level of AK027294 in NSCLC Tissues and Cells

Firstly, the AK027294 expression in 50 NSCLC tissues and 3 cell lines was detected *via* RT-qPCR. The results showed that AK027294 in NSCLC tissue samples was significantly up-regulated (Figure 1A). The AK027294 expression level in NSCLC cells was significantly higher than that of 16HBE cells (Figure 1B).

Silence of AK027294 Suppressed the Proliferation of NSCLC Cells

In this study, the H358 NSCLC cells were chosen for the silence of AK027294. Subsequently, the AK027294 expression in cells was detected by RT-qPCR (Figure 2A). The results of CCK8 assay showed that silence of AK027294 significantly inhibited the growth ability of NSCLC cells (Figure 2B). Meanwhile, colony formation assay revealed that the number of formed colonies was remarkably decreased after AK027294 silence in NSCLC cells (Figure 3A). Moreover, EdU incorporation assay indicated that the percentage of EdU positive cells was remarkably reduced after silencing AK027294 in H358 cells (Figure 3B).

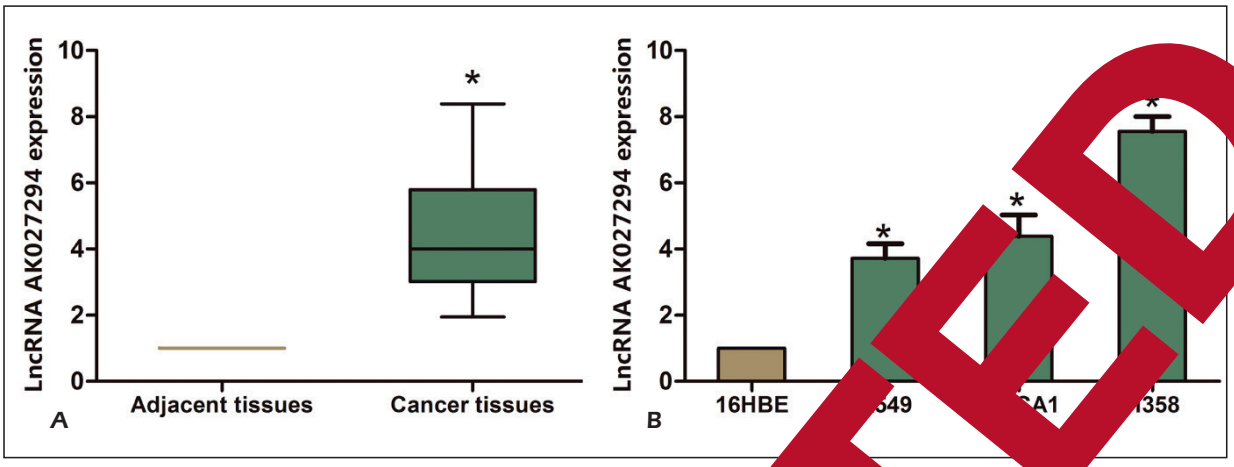


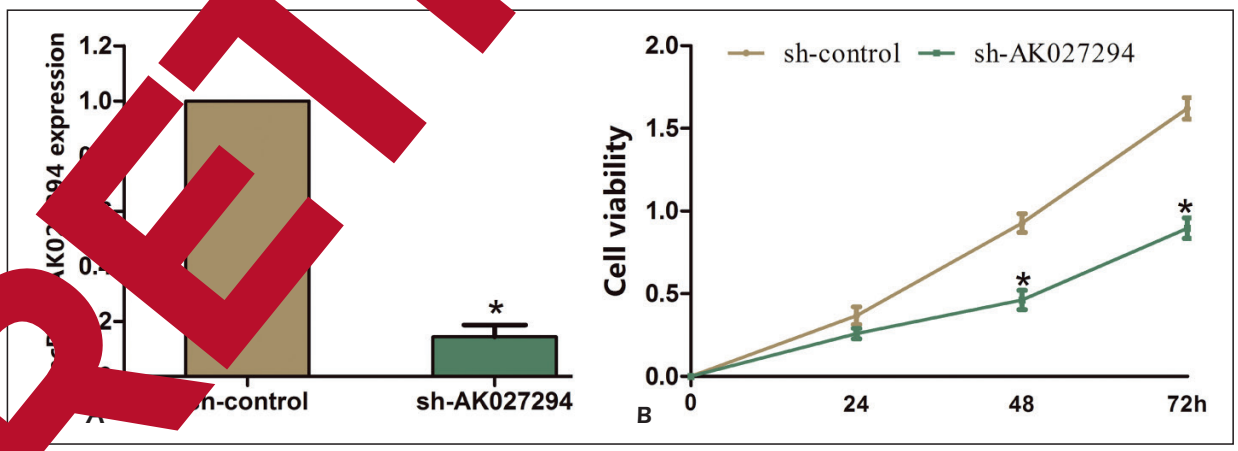
Figure 1. Expression levels of AK027294 were significantly increased in NS tis cell lines. **A**, AK027294 expression was significantly increased in NSCLC tissues when compared with adj nt tissue pression levels of AK027294 relative to GAPDH in human NSCLC cell lines and 16HBE (normal human bronchial epith l line) were determined by RT-qPCR. Data were presented as mean ± standard error of the mean.

Interaction Between STAT3 and AK027294 in NSCLC

RT-qPCR results showed that the expression level of STAT3 in NSCLC cells of sh-AK027294 group was remarkably lower than that of c l group (Figure 4A). Besides, we found STAT3 expression in NSCLC cells was fi- cantly increased when compared with 16 cells (Figure 4B). Furthermore, the results sho that the STAT3 expression in NSCLC tissues w remarkably higher than that t tissues (Figure 4C). Furthermore, th rrelat nalysis demonstrated that the STA xpressi s pos- itively correlated with th 272 in NSCLC tissues (Fi 4D

iscussion

RNAs a mportant regulators in lung can e and progression. For exam- ple, lncR HEIH facilitates the metastasis and roliferation of NSCLC cells, which may help to f r novel therapeutic intervention[10]. Ln- RNCR1 functions as an oncogene in NS- CLC, which promotes tumor progression through regulating miR-488/HEY2 signal network[11]. By sponging miR-27b-3p, lncRNA KCNQ1OT1 pro- motes the proliferation and invasion of NSCLC progression through up-regulating the expression of HSP90AA1[12]. In addition, overexpression of lncRNA-p21 suppresses the apoptosis of NSCLC



e 2. Silence of AK027294 repressed NSCLC cell proliferation. **A**, AK027294 expression in NSCLC cells transfected w AK027294 and control vector was detected by RT-qPCR. GAPDH was used as an internal control. **B**, CCK8 assay show at silence of AK027294 significantly repressed the proliferation of NSCLC cells. The results represented the average of three independent experiments (mean ± standard error of the mean). *p<0.05, as compared with control cells.

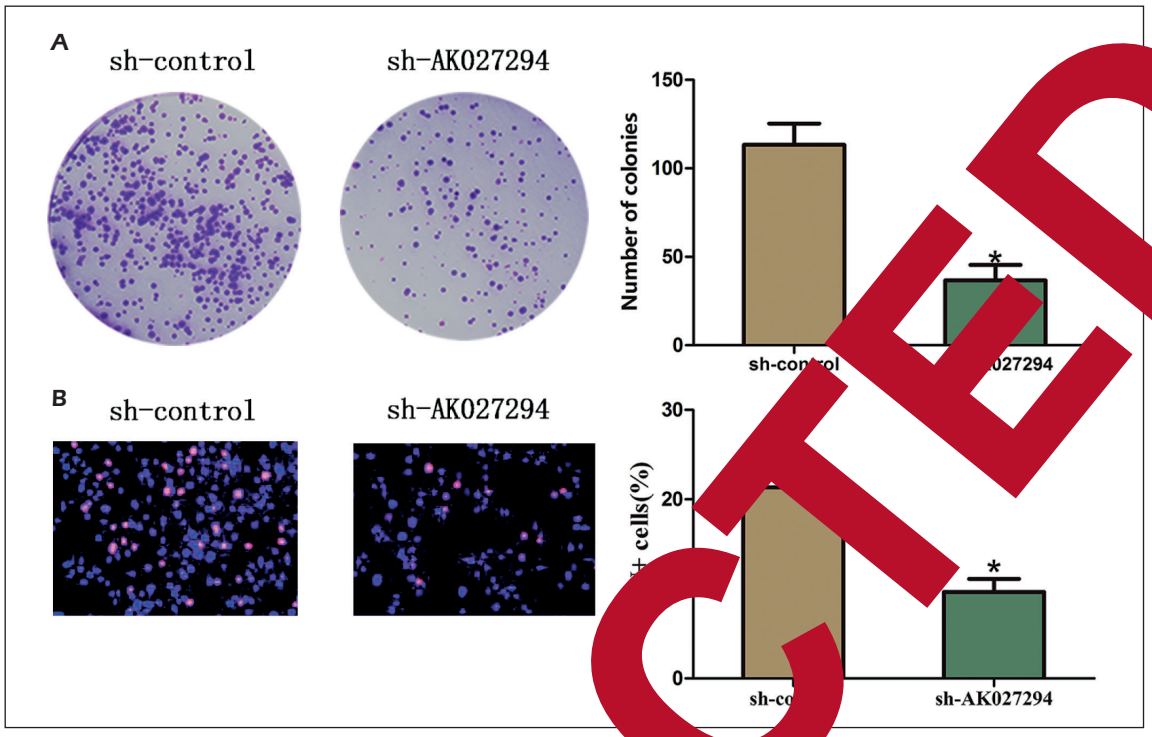


Figure 3. Silence of AK027294 inhibited NSCLC cell proliferation. A ation assay showed that the number of colonies was significantly decreased after silencing 27294 in NSCLC ce , EdU incorporation assay showed that the number of EdU positive cells was significantly de encing AK027294 in NSCLC cells. The results represented the average of three independent experiments (mea and e mean). *p<0.05, as compared with control cells.

cells by directly down-regulating the expressio level of PUMA[13].

LncRNA AK027294, as el mo lar, has been found to participate i cell cy rocess. Meanwhile, it is involved DN of colorectal cancer gre promoting cell proliferation a igration found that AK027294 was si antly up-reg both in NSCLC tissue nes. Besides, ence of AK027294 m edly d the proliferation of NSCLC ls. The abov lts indicated that AK027 promoted the tu genesis of NSCLC, h mig act as an oncogene.

3 is a ation of the signal transducer and t trans ion (STAT) factors, which h sho o be expressed in various cell ty [16] example, the STAT3 signaling pathwa esses the growth and invasive s of bladder cancer cells *via* inhibiting W ssion[17]. The IL-6/JAK/STAT3 way pla a crucial role in the progression colorectal cancer, which may help to offer tial therapeutic approaches[18]. MicroRNA functions as an oncogene in the progression of prostate tumor through impairing ubiquitination and activating STAT3[19]. Moreover, miR-124 suppresses NSCLC growth and induces cell apoptosis through negatively regulating STAT3 expression[20]. In the present work, we firstly verified the interaction between STAT3 and AK027294. The results showed that the expression level of STAT3 was significantly down-regulated after silencing AK027294. Besides, STAT3 expression was remarkably elevated in NSCLC cell lines. Furthermore, STAT3 expression in NSCLC tissues was positively correlated with AK027294 expression. All the results above suggested that AK027294 might promote the tumorigenesis of NSCLC through up-regulating STAT3.

Conclusions

We showed that AK027294 was remarkably up-regulated in NSCLC tissues. Meanwhile, it could facilitate the proliferation of NSCLC cells through up-regulating STAT3. These findings suggested that AK027294 might act as a candidate target for the treatment of NSCLC.

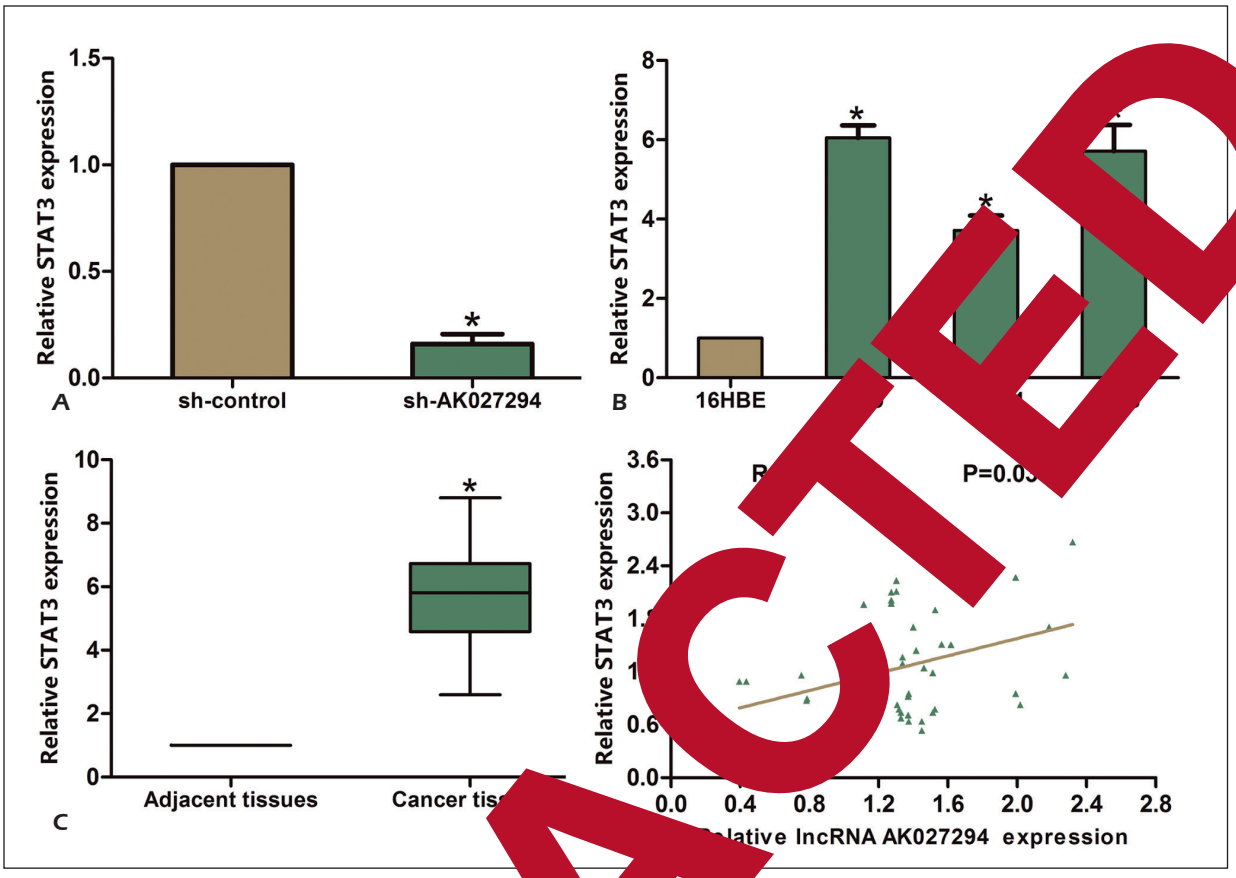


Figure 4. Interaction between AK027294 and STAT3. **A**, qRT-PCR results showed that STAT3 expression was significantly decreased in sh-AK027294 group when compared with sh-control group. **B**, Expression levels of STAT3 relative to GAPDH in human NSCLC cell lines and 16HBE (human bronchial epithelial cell line) were determined by RT-qPCR. **C**, STAT3 was significantly up-regulated in NSCLC tissues when compared with adjacent tissues. **D**, Linear correlation between the expression level of STAT3 and AK027294 in NSCLC tissues. The results represented the average of three independent experiments. Data were presented as mean ± standard error of the mean. *$p<0.05$.

Funding Acknowledgements

This work was supported by the Suzhou Science and Technology Project (SYS201862).

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.
2) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.
3) Chen W, Zheng R, Zeng H, Zhang S, He J. Annual report on status of cancer in China, 2011. Chin J Cancer Res 2015; 27: 2-12.
4) Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.
5) Xing Z, Wang HY, Su WY, Liu YF, Wang XX, Zhan P, Lv TF, Song Y. Wnt3 knockdown sensitizes human non-small cell type lung cancer (NSCLC) cells to cisplatin via regulating the cell proliferation and apoptosis. Eur Rev Med Pharmacol Sci 2018; 22: 1323-1332.
6) Chen X, Gao G, Liu S, Yu L, Yan D, Yao X, Sun W, Han D, Dong H. Long noncoding rna pvt1 as a novel diagnostic biomarker and therapeutic target for melanoma. Biomed Res Int 2017; 2017: 7038579.
7) Cheng S, Li T, Wang C, Wang K, Lai C, Yan J, Fan H, Sun F, Wang Z, Zhang P, Yu L, Hong Z, Lei G, Sun B, Gao Y, Xiao Z, Ji X, Wang R, Wu J, Wang X, Zhang S, Yang P. Decreased long intergenic noncoding RNA P7 predicts unfavorable prognosis and promotes tumor proliferation via the modulation of the STAT1-MAPK pathway in hepatocellular carcinoma. Oncotarget 2018; 9: 36057-36066.
8) Tang LX, Chen GH, Li H, He P, Zhang Y, Xu XW. Long non-coding RNA OGFRP1 regulates LYPD3 expression by sponging miR-124-3p and promotes non-small cell lung cancer progression. Biochem Biophys Res Commun 2018; 505: 578-585.

9) Zhou X, Chen H, Zhu L, Hao B, Zhang W, Hua J, Gu H, Jin W, Zhang G. Helicobacter pylori infection related long noncoding RNA (lncRNA) AF147447 inhibits gastric cancer proliferation and invasion by targeting MUC2 and up-regulating miR-34c. Oncotarget 2016; 7: 82770-82782.
10) Jia K, Chen F, Xu L. Long noncoding RNA HEIH promotes the proliferation and metastasis of non-small cell lung cancer. J Cell Biochem 2018.
11) Cheng D, Bao C, Zhang X, Lin X, Huang H, Zhao L. LncRNA PRNCR1 interacts with HEY2 to abolish miR-448-mediated growth inhibition in non-small cell lung cancer. Biomed Pharmacother 2018; 107: 1540-1547.
12) Dong Z, Yang P, Qiu X, Liang S, Guan B, Yang H, Li F, Sun L, Liu H, Zou G, Zhao K. KCNQ1OT1 facilitates progression of non-small-cell lung carcinoma via modulating miRNA-27b-3p/HSP90AA1 axis. J Cell Physiol 2018.
13) Yang T, Zhang W, Wang L, Xiao C, Guo B, Gong Y, Liang X, Huang D, Li Q, Nan Y, Xiang Y, Shao J. Long intergenic noncoding RNA-p21 inhibits apoptosis by decreasing PUMA expression in non-small cell lung cancer. J Int Med Res 2018: 1218616144.
14) Niu H, Hu Z, Liu H, Hu G, Yang B, Wu S, Li F. Long non-coding RNA AK027294 involves in the process of proliferation, migration, and apoptosis of colorectal cancer cells. Tumour Biol 2016; 37: 10097-10105.
15) Uzel G, Sampaio EP, Lawrence MG, Hsu AP[illegible] M, Dorsey MJ, Noel RJ, Verbsky JW, Free[illegible]AF, Janssen E, Bonilla FA, Pechacek J, Chandras[illegible]n P, Browne SK, Agharahimi A, Gharib AM, Mann[illegible] SC, Yim JJ, Gambineri E, Torgerson T, Tran DQ, Milner JD, Holland SM. Dominant gain-of-function STAT1 mutations in FOXP3 wi[illegible] mune dysregulation-polyendo[illegible] teropathy-X-linked-like syndrome[illegible]llergy Cli[illegible] Immunol 2013; 131: 1611-1623[illegible]
16) Holland SM, DeLeo FR, Elloum[illegible]su AP, Uzel G, Brodsky N, Freeman AF, Dem[illegible]A, Davis J, Turner ML, Anderson [illegible] Darnell[illegible]Welch PA, Kuhns DB, Frucht [illegible] Malech HL, [illegible] Kobayashi SD, Whitney[illegible] Voyich JM, Muss[illegible] Woellner C, Schaff[illegible], Puck[illegible] Grimbacher [illegible]. STAT3 mutations[illegible]e hyp[illegible]E syndrome. N Engl J Med 2007; [illegible]9.
17) Tsujita Y, Hori[illegible]A, [illegible]sono M[illegible]o T, Ito K, Asano T[illegible]mi Y, Kush[illegible] STAT[illegible]ibition by WP1066 [illegible]sses the grow[illegible]asiveness of bladder[illegible]lls. Oncol Rep 2[illegible]8: 2197-2204.
18) Wang [illegible], S[illegible]. The IL-6/JAK/STAT3 pathway: [illegible]tential t[illegible]utic strategies in treating colorectal cancer ([illegible]). Int J Oncol 2014; 44: [illegible]40.
[illegible]allavalle C, Albino D, Civenni G, Merulla J, Ostano P, Mello-Grand M, Rossi S, Losa M, D'Ambrosio G, Sessa F, [illegible]mann GN, Garcia-Escudero R, Zitella A, Chi[illegible]o G, Catapano CV, Carbone GM. [illegible]icroRNA-4[illegible]mpairs ubiquitination to activate [illegible]T3 and [illegible]mote prostate tumor progression. [illegible]016; 126: 4585-4602.
20) Wang [illegible]eng B, Liu Y, Yu J, Chen Q, Liu Y. MiR-124 inhibits growth and enhances radiation-in[illegible]ed apoptosis in non-small cell lung cancer by [illegible]ing STAT3. Cell Physiol Biochem 2017; 44: 20[illegible]-2028.