2018; 22: 8599-8608

RETRACTED

Targeted regulation of miR-195 on MAP2K1 for suppressing ADM drug resistance in prostate cancer cells

J.-Y. ZHANG, Y.-N. LI, X. MU, Z.-L. PAN, W.-B. LIU

Department of Urology, The Second Affiliated Hospital, Fujian Medical [illegible]ersity, [illegible]anzhou, Fujian, China

Abstract. – **OBJECTIVE:** Extra-cellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) signaling pathway participates in cell proliferation, cycle and apoptosis. MAPK kinase 1 (MAP2K1) activates the ERK/MAPK pathway. The down-regulation of miR-195 is correlated with the onset and drug resistance of prostate cancer. Bioinformatics analysis identified complementary binding sites between miR-195 and MAP2K1. This study aimed to investigate the effect of miR-195 on the [illegible]eration, apoptosis and adriamycin (ADM[illegible] tance of prostate cancer cells.

MATERIALS AND METHODS: Dual-[illegible]ferase reporter gene assay confirmed ta[illegible]d regulation between miR-195 and MAP2K1. [illegible] resistant cell line DU145/ADM and PC-3/A[illegible] were generated for comparing [illegible]R-195 an[illegible] MAP2K1 expression. Apopt[illegible]easured by flow cytometry and ca[illegible]se-3 a[illegible]ity was quantified. Cultured cells [illegible]re treate[illegible]ith miR-195 mimic, followed by [illegible]titati[illegible] PCR (qRT-PCR) was [illegible]sed [illegible]sion. Western blot [illegible]asured [illegible]K1, ERK1/2 and phosphorylat[illegible] ERK1/2 (p-E[illegible]) expression, and flow [illegible]try quantifie[illegible] apoptosis, followed [illegible]aining for cel[illegible] proliferation.

RESULTS: Targeted [illegible]ion existed between mi[illegible]95 and MAP2K[illegible]A. Drug-resistant ce[illegible]ad lower miR-195 th[illegible] parental cells, whils[illegible]AP2K1 expression was higher. Under ADM[illegible]atment [illegible]h IC50 concentration, drug resista[illegible]wed low[illegible] apoptosis. The transfection [illegible]-195 de[illegible]ased MAP2K1 expres[illegible] and [illegible]/2, [illegible]ated cell apoptosis and [illegible]ssed [illegible]tive rate or cell proliferatio[illegible]

C[illegible]CLUSIONS: The down-regulation of miR-195 [illegible]ted with ADM resistance of pros[illegible]ls. The over-expression of miR-[illegible] weakens cancer cell proliferation, facilitates [illegible]poptosis and decreases ADM resistance vi[illegible]ted inhibition on MAP2K1 expression and [illegible]/MAPK signal pathway.

Key Wor[illegible]

Prostate cancer, [illegible]5, MAP2K1, ERK/MAPK, Drug resistance.

[illegible]roduction

[illegible]tate carc[illegible]ma is a common malignant tum[illegible]e urinal-reproductive system. Due to its [illegible]atively high incidence, it is the sixth [illegible]st popular malignant tumor in males and has [illegible]igher frequency in male urinal repro[illegible]ctiv[illegible]umors[1,2]. Prostate cancer has relative[illegible]y higher difficulty for treatment, unfavorable prognosis and higher mortality. Chemotherapy has become a critical treatment measure for prostate cancer, whilst the drug resistance is one major factor limiting clinical treatment efficiency[3,4]. Mitogen-activated protein kinase kinase 1 (MAP2K1) is the upstream protein kinase of extra-cellular signal-regulated kinase (ERK) and can activate extra-cellular signal regulated kinase/mitogen activated protein kinase (ERK/MAPK) signal pathway. Abnormally elevated expression and function of MAP2K1 are correlated with onset, progression, metastasis and drug resistance of various tumors[5-7]. Previous studies showed the involvement of enhanced MAP2K1 expression or functional activity in malignant features including drug resistance and invasion of prostate cancer[8,9]. MicroRNA (miR) is an endogenous non-coding small RNA molecule in eukaryotes and can regulate target gene expression by degrading mRNA or inhibiting mRNA translation by complementary binding with 3'-untranslated region (3'-UTR) of target gene mRNA, thus participating in the regulation of biological processes including cell survival, proliferation, apoptosis and migration.

Corresponding Author: Yining Li, MD; e-mail: jiaoyuanshan93@163.com

RETRACTED

Abnormal expression or function of microRNA has attracted increasing interests in tumor drug resistance[10,11]. Previous studies[12-14] showed the correlation between miR-195 down-regulation and the onset, progression, prognosis and drug resistance of prostate cancer. Bioinformatics analysis showed the existence of complementary binding sites between miR-195 and 3'-UTR of MAP2K1 mRNA, indicating possibly targeted regulatory roles. This work thus investigated the role of miR-195 in mediating MAP2K1 expression and affecting proliferation, apoptosis and ADM resistance of prostate cancer cells.

Materials and Methods

Major Reagents and Materials

Normal prostate gland epithelial cell RWPE-1 (CRL-11609), prostate cancer cell line DU145 (HTB-81) and PC-3 (CRL-1435) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Dulbecco's Modified Eagle Medium (DMEM), keratinocyte-serum free medium (keratinocyte-SFM), [illegible] minimum essential media (Opti-MEM) [illegible]tal bovine serum (FBS) were purchase[illegible]om Gibco (Grand Island, NY, USA). RNAis[illegible]s was purchased from TaKaRa (Otsu, Shiga[illegible]pan). Fluorescent quantitative PCR kit Trans[illegible]cript Green One-Step quant[illegible]al Time PCR (qRT-PCR) SuperMix [illegible]s pur[illegible]ed from Transgen (Beijing, China[illegible]iR-195 [illegible]mic and microRNA-normal cont[illegible]iR-NC[illegible]chased from Ribobio (Guan[illegible]). [illegible]fectamine 2000 wa[illegible]urchase[illegible] Invitrogen (Carlsbad, CA, U[illegible]). Rabbit ant[illegible]n polyclonal antibody [illegible] MAP2K1, E[illegible]/2 and p-ERK1/2 w[illegible]pur[illegible] from Abcam (Cambridge, MA, USA). Ra[illegible]ti-human β-actin polyclon[illegible]ntibody was pu[illegible]ed from Santa Cruz [illegible]echnology (Santa C[illegible]z, CA, USA). Hors[illegible]dish pe[illegible]xidase (HRP) conjugated secon[illegible]ntib[illegible] was purchased from Sangon Biote[illegible]td. (Sh[illegible]ai, China). Annexin V/propidi[illegible]dide [illegible] cell apoptosis test kit [illegible]eyoE[illegible] chemiluminescence were pu[illegible]ed from [illegible]yotime (Shanghai, China). Adr[illegible]ycin (ADM) and cell counting kit were pu[illegible] MedchemExpress (Monmouth [illegible], USA). EdU cell proliferation flow [illegible]metry kit was purchased from Molecular [illegible] (Eugene, OR, USA). Luciferase activity assa[illegible]t Dual-Glo Luciferase Assay System, and Dual-Luciferase reporter plasmid pL[illegible] Luciferase vector were purchased fro[illegible] Inc. (Austin, TX, USA).

Cell Culture

DU145 and PC-3 cells we[illegible]bated in DMEM medium contain[illegible] 10% [illegible]bovine serum (FBS) and were[illegible]ept in a 3[illegible] cubator with 5% CO[illegible]odel HERAcell[illegible], Thermo Fisher Scie[illegible]c, Walt[illegible]n, MA, USA). RWPE-1 cells were[illegible]in[illegible]ratinocy[illegible]-SFM medium contai[illegible]g 5 [illegible] epitheli[illegible] growth factor (EGF) [illegible]d 0.0 mg[illegible]ovi[illegible] Pituitary Extract (BP[illegible] a 37° C incu[illegible]h 5% CO_2. Cells were[illegible] 1:4 ratio, and [illegible] cells at log-growth [illegible]se we[illegible]d for experiments.

Ge[illegible]ion of Adria[illegible]n (ADM) [illegible]stant Cell Model

To generate an ADM drug-resistant cell model, [illegible]145 and PC[illegible]ells at log-growth phase were [illegible]enished with [illegible]μg/ml ADM into the culture m[illegible]n. 24 h [illegible]r, the fresh culture medium was [illegible]ter 2 weeks of stable growth, the ADM concentration was gradually elevated to 2 [illegible]ml, 4 μg/ml, 8 μg/ml and 16 μg/ml until sta[illegible] for repeated passage within 16 μg/ml [illegible]DM. [illegible]DM resistant prostate cancer cell lines DU145/ADM and PC-3/ADM were then generated. DU145, PC-3, DU145/ADM and PC-3/ADM cells were seeded into 96-well plate at 10000 cells per well density. After 24 h attached growth, cells were treated with 0, 5, 10, 20, 40, 80, 160 and 320 μg/ml ADM, with 6 parallel replicates for each concentration. After 48 h incubation, each well was added with 10 μl cell counting kit-8 (CCK-8) solution, and absorbance (A) values at 450 nm wavelength (A450) were measured after 4 h reaction. Inhibition rate = (1-A450 of treatment group)/A450 of treatment group×100%. Half maximal inhibitory concentration (IC_{50}) for the concentration of inhibiting 50% cell growth was calculated by SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). Resistance index (RI) = IC_{50} of drug-resistant cells/IC_{50} of parental cells.

Flow Cytometry for Cell Proliferation

Cells were re-suspended in DMEM medium containing 10% FBS. EdU Alexa Fluor 488 Flow Cytometry Assay Kit (Cat. No. 35002, Invitrogen, Carlsbad, CA, USA) was used to measure proliferation potency of cells. In brief, cells were incubated using 10 μM EdU for 2 h and were continuously incubated for 48 h. Cells were digested

by trypsin and collected. After centrifugation, washing, fixation and permeabilization, cells were incubated in assay buffer containing Alex Fluor 488 labels for 30 min room temperature incubation. After washing and centrifugation, FC500 MCL flow cytometry (Beckman Coulter Inc., Brea, CA, USA) was used to measure cell proliferation.

Dual Luciferase Activity Assay

PCR products for full-length fragment or mutant form of MAP2K1 gene 3'-UTR were digested by dual restriction enzymes and were ligated to pLUC plasmid. After transforming competent bacteria, the cells with correct sequences were selected and named as pLUC-MAP2K1-WT or pLUC-MAP2K1-MUT. Lipofectamine 2000 was used to co-transfect pLUC-MAP2K1-WT (or pLUC-MAP2K1-MUT) and miR-195 mimic (or miR-NC) into HEK293T cells for 48h continuous incubation. Dual-Glo Luciferase Assay System kit was used for measuring Dual-Luciferase activity.

Cell Transfection and Grouping

Cultured DU145/ADM and PC-3/AD[illegible] c[illegible] were divided into two groups: miR-N[illegible]ns-fection group, and miR-195 mimic transf[illegible]n group. In brief, 100 µl serum-free medium [illegible] used to dilute 10 µl Lipofectamine 2000, [illegible] nmol miR-NC, 30 nmol miR-[illegible]. After [illegible] min room temperature incub[illegible]on, Li[illegible]ctamine 2000 was gently mixed w[illegible] miR-NC [illegible]nd miR-203 mimic for 20 min r[illegible]mpera[illegible]tion. The transfection mixt[illegible]wly [illegible]d into the culture me[illegible]m for ge[illegible]ixture and 82 h of continuou[illegible]ubation for c[illegible]g cells. Cells from all [illegible]ere seeded in[illegible] 6-well plate, and 50 [illegible]ml A[illegible]s added when reaching 50% confluence. A[illegible] h of continuous incubatio[illegible]ell apoptosis w[illegible]sured by flow cytome[illegible] Trypsin (Beyotime, [illegible]anghai, China) was [illegible]d to coll[illegible]t treated cells from all groups. Af[illegible]uM [illegible] incubation for 2 h, cells were continuously incubated for 48 h as descri[illegible] in previous sections. Cell proliferation [illegible] measured by test kit.

QRT-PCR for Measuring G[illegible] Expression

RNAiso Plus was used to [illegible] cellular RNA. The relative expr[illegible]ion of [illegible] gene was measured by qRT-[illegible]R using T[illegible] Green One-Step qRT-P[illegible] SuperMix. In a[illegible] reaction system, one[illegible]ded 1 µ[illegible]emplate RNA, 0.3 µM forward pri[illegible]3 [illegible] reverse [illegible]rimer, 10 µl 2× TransS[illegible]t Tip[illegible] qPCR S[illegible]erMix, 0.4 µl One-Ste[illegible]T Enzym[illegible] 0[illegible] Passive Reference D[illegible] and RNase-[illegible]er up to 20 µl. The q[illegible]action conditi[illegible]s were: 45° C 5 min a[illegible]4° C [illegible]ollowed by 40 cycles each consisting of 95° C [illegible] 60° C 30 s. The gene exp[illegible] was measu[illegible] Bio-Rad CFX96 R[illegible]e fluorescent quantitative PCR cycler [illegible]io-Rad, Hercules, CA, USA). The primers for [illegible] PCR were li[illegible] in Table I.

W[illegible]rn Blot

P[illegible] extracted by radioimmunoprecipitation assay (RIPA) lysis buffer (Tiangen [illegible]tech Co. Ltd., Beijing, China), followed by [illegible]ion and quality control. Total of 40 µg [illegible] were separated in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (10% separating gel, 4% condensing gel, 45 V, 150 min) and were transferred to polyvi-[illegible]ylidene difluoride (PVDF, Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) membrane (300 mA, 100 min). The membrane was blocked in 5% defatted milk powder at room temperature and was incubated in primary antibody (MAP2K1 at 1:2000, ERK1/2 at 1:2000, p-ERK1/2 at 1:1000, and β-actin at 1:10000) for 4°C overnight incubation. On the next day, the membrane was washed in Phosphate-Buffered Saline and Tween-20 (PBST) three times, and horseradish peroxidase (HRP) conjugated secondary antibody (1:15000 dilution) was added for 60 min room temperature incubation, followed

Table I. Pri[illegible] the [illegible] assay.

[illegible]ene		Sequences
mi[illegible]95	Forwards	5'-GAATCCGCCTCAAGAGAACAAGGTGGAG-3'
	Reverse	5'-AGATCTCCCATGGGGGCTCAGCCCCT-3'
[illegible]AP2K1	Forwards	5'-ATCTTCGGGAGAAGCACAAG-3'
	Reverse	5'-CGAAGGAGTTGGCCATAGAG-3'
[illegible]	Forwards	5'-TACCACATCCAAGAAGGCAG-3'
	Reverse	5'-TGCCCTCCAATGGATCCTC-3'

RETRACTED

RETRACTED

by washing in PBST three times. BeyoECL Plus working solution (prepared from an equal volume of solution A and B) was added onto the membrane. After 2-3 min of dark incubation, the membrane was exposed, and images were scanned for data processing.

Cell Apoptosis Assay

Cells were digested by Trypsin and collected for Phosphate-Buffered Saline (PBS, Tiangen Biotech Co. Ltd., Beijing, China) washing by centrifugation. Cells were resuspended in 100 μl Annexin V Binding Buffer, and 5 μl Annexin V-FITC and 5 μl PI were sequentially added for staining. After 15 min of room temperature incubation, 400 μl Annexin V Binding Buffer was added for measuring cell apoptosis on FC500 MCL flow cytometry (Beckman Coulter, Brea, CA, USA).

Statistical Analysis

SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA) was used for data processing. Measurement data were presented as mean ± standard deviation (SD). The Student's *t*-test was used to compare the differences between the two g[illegible] The differences among groups were anal[illegible] using Tukey's post-hoc test to validate t[illegible]ne-way analysis of variance (ANOVA). A sta[illegible]l significance was defined when $p < 0.05$.

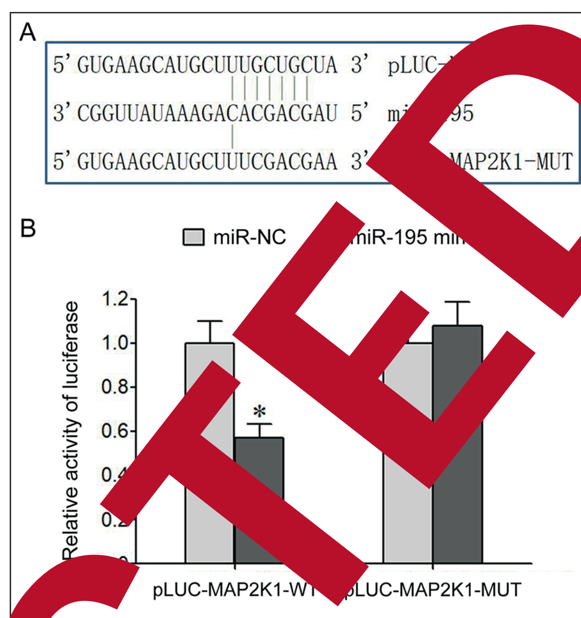
Result[illegible]

Targeted Regulation [illegible]een [illegible] and MAP2K1 MRNA

Bioinformatics a[illegible]ysis sho[illegible] existence of complementary [illegible]ding sites be[illegible]iR-195 and 3'-UTR of [illegible]K1 mRNA (F[illegible]re 1A). Dual-Lucifer[illegible] gene[illegible]ter assay showed that the transfection of miR-1[illegible]ic remarkably decreased r[illegible]ive Luciferase a[illegible]y in HEK293T cells tr[illegible]ected with pLUC-M[illegible]P2K1-WT plasmid[illegible]lst had [illegible]o significant effect on the relativ[illegible]iferas[illegible]tivity of HEK293T cells transfected [illegible] pLUC-[illegible]P2K1-MUT plasmid (Figure 1[illegible]se re[illegible]ts suggest that miR-195 [illegible]get 3'-[illegible]AP2K1 mRNA to inhibit its [illegible]ession.

Do[illegible]on of MiR-195 and [illegible]egula[illegible]n of MAP2K1 in Drug [illegible]istant Prostate Cancer Cell Lines

[illegible]ental DU145 cells, IC_{50} reached 7.44±0.82 μg/m[illegible] whilst drug-resistant DU145/ADM cells



[illegible]ure 1. Targe[illegible] regulation between miR-195 and [illegible]2K1 mRNA. *A*[illegible]nctional site between miR-195 and 3[illegible] MAP2K1 m[illegible]A. ***B***, Dual-Luciferase gene reporter ass[illegible]0.05 co[illegible]red to the miR-NC group.

[illegible]d IC_{50} values at 86.37±7.11 μg/ml. The relative [illegible]tant index of DU145/ADM cells against [illegible]U145 cells was 11.61 (Table II). Parental PC-3 cells had IC_{50} values at 5.29 ± 0.47 μg/ml, and drug-resistant cell line PC3/ADM had IC_{50} values at 57.71±5.35 μg/ml, with relative drug-resistant index at 10.91 against parental cells (Table I). The qRT-PCR results showed that, compared to normal prostate epithelial cell line RWPE-1, DU145 cells showed significantly decreased miR-195 expression, and even lower miR-195 expression occurred in drug-resistant cell line DU145/ADM cells. PC-3 cells had remarkably lower miR-195 expression compared to RWPE-1 cells, and drug-resistant cell PC-3/ADM had an even lower miR-195 expression (Figure 2A). Compared to RWPE-1 cells, DU145 cells had significantly higher MAP2K1 mRNA expression, which was further elevated in drug-resistant DU145/ADM cells. PC-3 cells also showed higher MAP2K1 mRNA expression compared to RWPE-1 cells, and MAP2K1 mRNA level was even higher in drug-resistant PC-3/ADM cells (Figure 2B). Western blot results showed that, compared to RWPE-1 cells, DU145 cells had remarkably higher MAP2K1 proteins, which was further elevated in drug-resistant DU145/ADM cells. Moreover, compared to RWPE-1 cells, PC-3 cells showed remarkably elevated MAP2K1 protein expres-

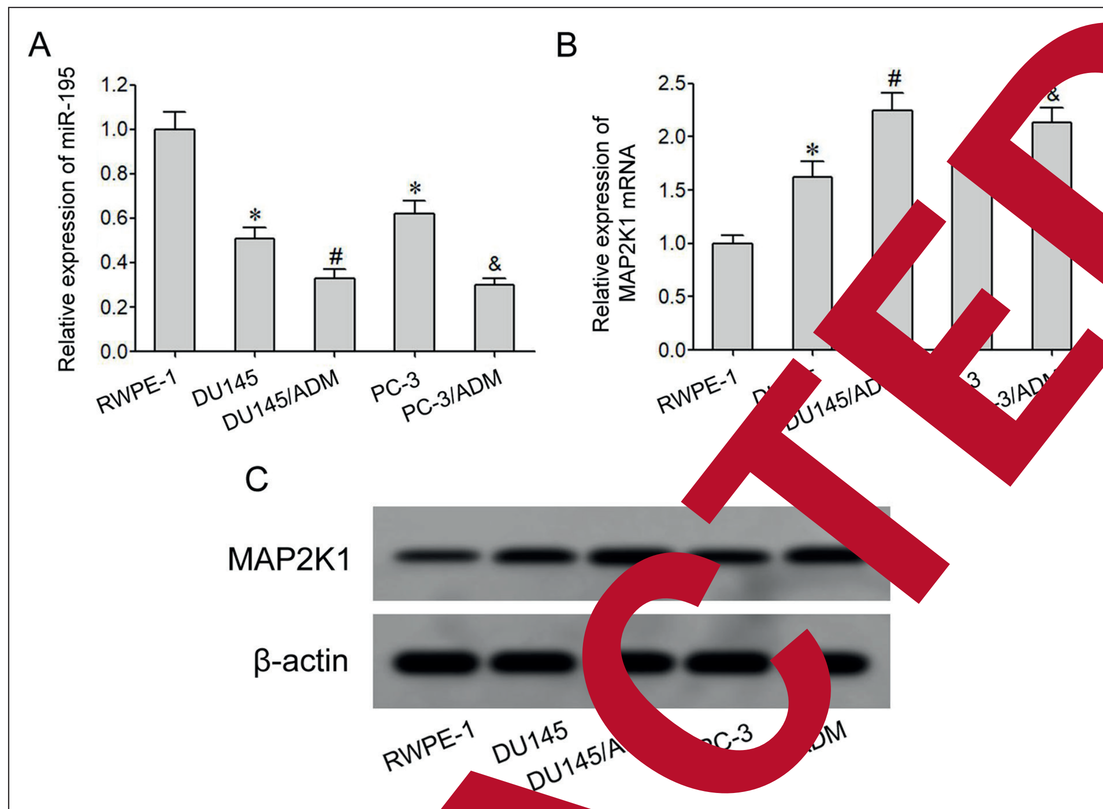


Figure 2. Down-regulation of miR-195 and up-re[illegible] drug-resistant prostate cancer cell line. ***A,*** qRT-PCR for miR-195 expression. ***B,*** qRT-PCR measuring M[illegible]1 mRNA[illegible] ***C,*** Western blot for protein expression. $^{*}p < 0.05$ compared to RWPE-1 cells, $^{\#}p < 0.05$ compared to D[illegible] cells; [illegible] 0.05 [illegible]mpared to PC-3 cells.

RETRACTED

sion, and drug-resistant cell PC[illegible] had muc[illegible] higher MAP2K1 protein lev[illegible] (Figu[illegible] C).

Drug Resistant Cells [illegible]nted Resistance Agains[illegible] ADM[illegible] Cell Apoptosis

Under the trea[illegible]nt of ADM w[illegible] concentrations equivalen[illegible] values of D[illegible]5 cells (7.51 μg/ml), [illegible]145 [illegible] presented prominent cell apoptosis, whilst [illegible]5/ADM cells had relatively [illegible]wer apoptosis ([illegible] 3A) plus significan[illegible] depressed caspase-3 activity (Figure 3B). [illegible]ng 5.29 [illegible]g/ml ADM equivalent to IC_{50}, PC[illegible] pre[illegible]ted prominent cell apoptosis, whilst [illegible]M cell h[illegible] relatively fewer apoptosis (Fig[illegible]C) plu[illegible] ower caspase-3 activity ([illegible]e 3D).

Ov[illegible]xpression of MiR-195 can Suppress Dr[illegible]ce of Prostate Cancer Cells [illegible] Down[illegible]gulating MAP2K1

[illegible]nder treatment of 16 μg/ml ADM, both D[illegible]ADM and PC-3/ADM cells presented extr[illegible]ly lower apoptotic rates (Figure 4B) whilst proliferation potency was fruitful (Figure 4C). The qRT-PCR results showed that the transfection of miR-195 mimic into DU145/ADM and PC-3/ADM cells remarkably decreased the MAP2K1 protein expression compared to miR-NC group, and p-ERK1/2 protein expression was remarkably suppressed (Figure 4B). Flow cytometry results showed that the transfection of miR-195 mimic remarkably elevated apoptosis of DU145/ADM and PC-3/ADM cells under 16 μg/ml Adm treatment (Figure 4D), whilst cell proliferation potency was significantly depressed (Figure 4E, F).

Discussion

Prostate cancer is the malignant tumor originated from prostate gland epithelium. Having relatively higher malignancy and mortality rate, prostate cancer is the second popular cancer in males, only lower than the most deadly pulmonary carcinoma[15,16]. Although China has a relatively lower incidence of prostate cancer than

RETRACTED

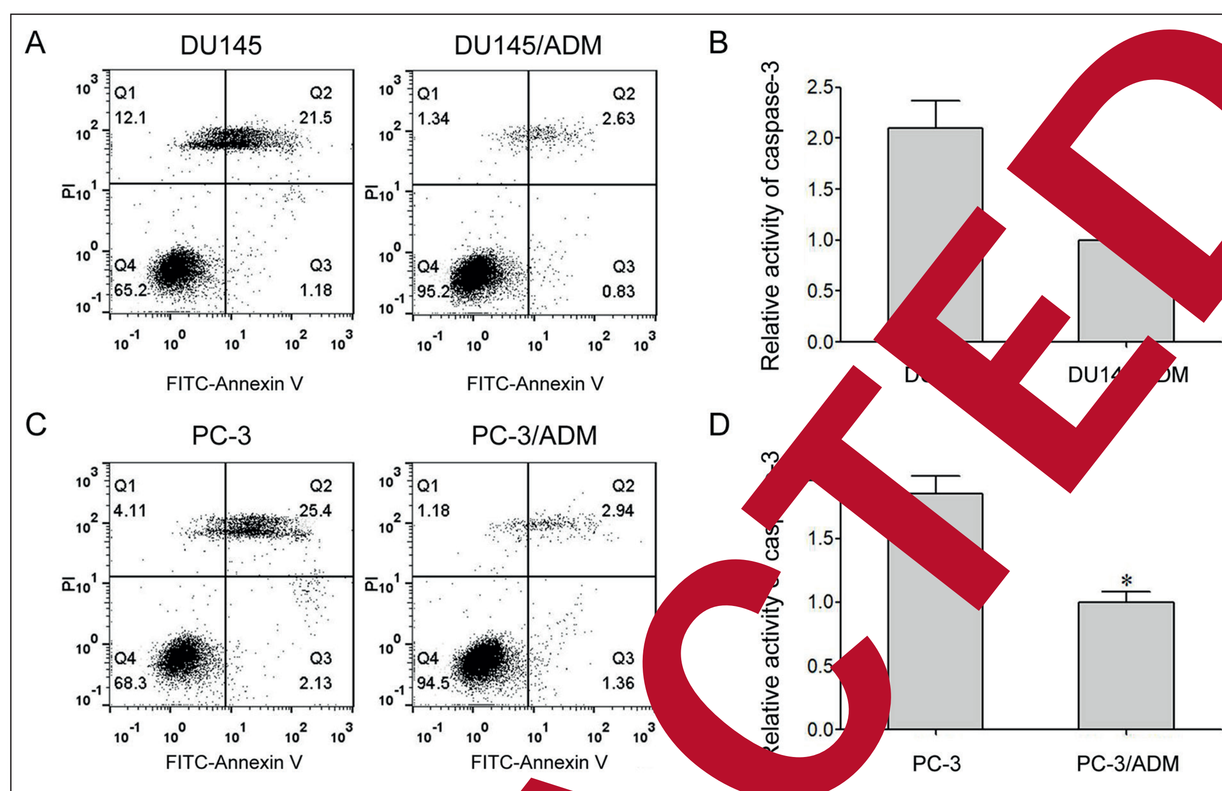
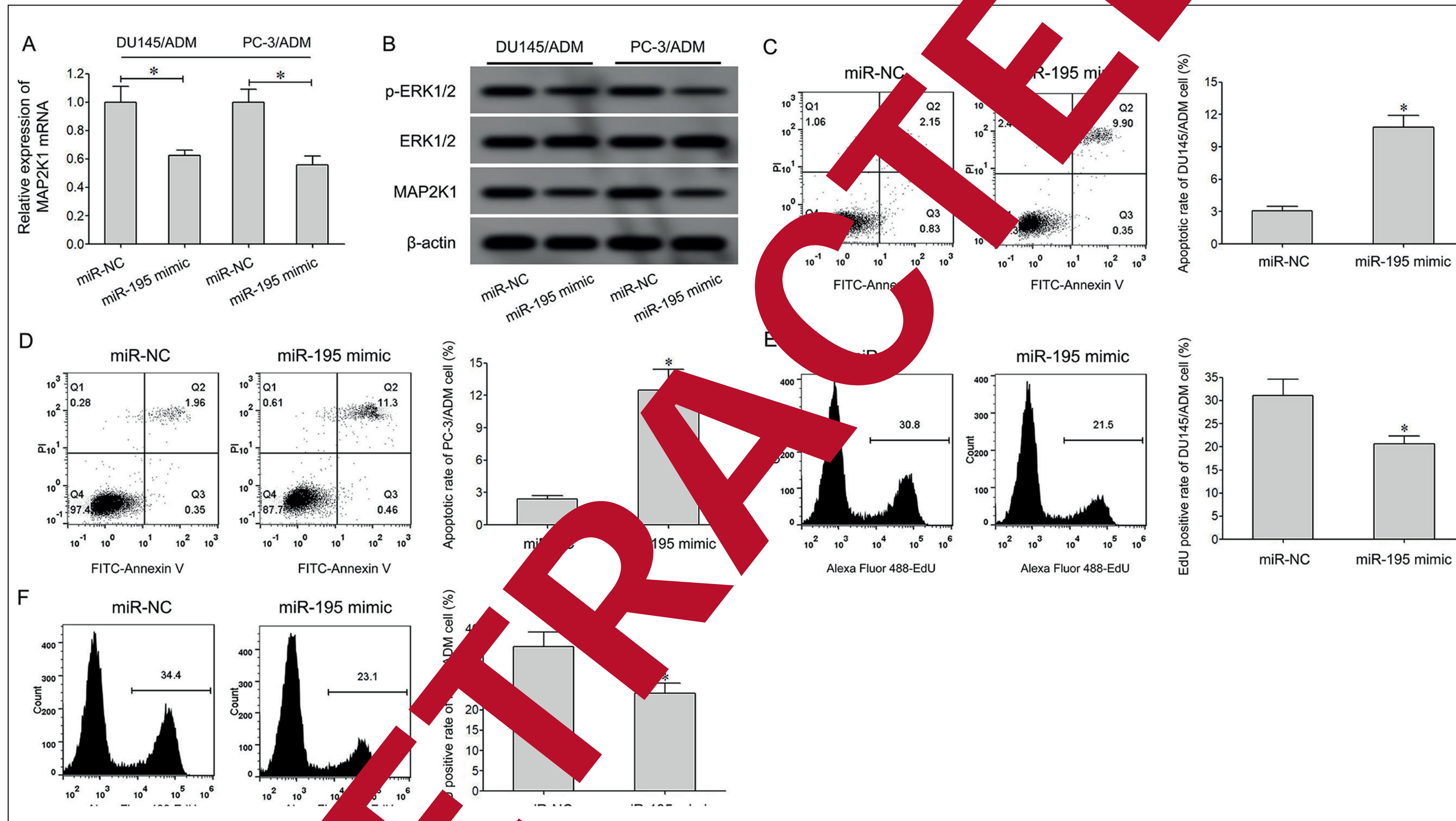


Figure 3. Drug-resistant cells presented resista[illegible] ga[illegible]ced cell apoptosis. ***A,*** Flow cytometry for DU145 and DU145/ADM cell apoptosis. ***B,*** Comparison of ap[illegible]s rate be[illegible] and DU145/ADM cells. ***C,*** Flow cytometry for apoptotic rate of PC-3 and PC-3/ADM cell. ***D,*** Statis[illegible]nalysi[illegible] ap[illegible]ic rates between PC-3 and PC-3/ADM cells. $^{*}p < 0.05$ comparing the two groups.

Western countries, its incid[illegible]e is p[illegible]essively increasing due to populati[illegible] aging a[illegible] lifestyle transition[17,18]. Chemothe[illegible] the m[illegible] for treating prostate [illegible]arcin[illegible] drug [illegible]tance has become th[illegible] major fac[illegible]cting treatment efficiency, l[illegible]ng to chemot[illegible] failure and unfavorable [illegible]is. Therefore, [illegible] investigation for m[illegible]ular [illegible]idates with abnormal change during chemothe[illegible]sistance is of critical imp[illegible]nce for revealin[illegible] mechanism for drug re[illegible]ance, suppressing dr[illegible] resistance, improv[illegible] treatme[illegible] efficiency, guiding individualize[illegible]ment [illegible]d improving survival and prognosis. [illegible]un N-te[illegible]nal kinase (JNK) and p38 and [illegible]/big M[illegible]P kinase 1 (BMK1) are [illegible]ajor tr[illegible] pathways for MAPK signa[illegible]way. Am[illegible] those, ERK-induced MAPK sign[illegible] transduction pathway is the classical M[illegible]ansduction pathway, and is the [illegible] tra[illegible]uction pathway through which [illegible]PK signal pathway exerts its roles[19-21]. The E[illegible]APK signal transduction pathway is wide[illegible] expressed in various tissues and cells and can regulate various biological processes including cell proliferation, apoptosis and invasion[22-25]. It is closely correlated with tumor pathogenesis, progression and drug resistance[26-28]. MAP2K1 is a dual-specific protein kinase that exerts functions upstream of ERK protein and can phosphorylate tyrosine/threonine residues of substrate ERK protein, thus activating the ERK/MAPK signal pathway[29,30]. Previous studies[8,9] showed that enhanced expression or function of MAP2K1 are correlated with malignant properties of prostate cancer such as drug resistance and invasion. MiR-195 is a widely studied microRNA molecule, and its abnormal expression plays roles in the onset and progression of multiple tumors including lung cancer[31], colorectal carcinoma[32], and pancreatic cancer[33]. Previous studies[12-14] demonstrated the correlation between miR-195 down-regulation and the onset, progression, prognosis and drug resistance of prostate cancer. Bioinformatics analysis showed the complementary binding sites between miR-195 and 3'-UTR of MAP2K1 mRNA, suggesting potentially tar-



RETRACTED

Figure 4. Over-expression of miR-195 dec drug res nce of prostate cancer cell by down-regulating MAP2K1. ***A,*** qRT-PCR for measuring MAP2K1 mRNA expression. ***B,*** Western blot for protein expre ***C,*** Flow or apoptosis of DU145/ADM cells. ***D,*** Flow cytometry for PC-3/ADM cell apoptosis. ***E,*** Flow cytometry for DU145/ADM cell proliferation. ***F,*** Flo for PC cell proliferation. $^{*}p < 0.05$ comparing the two groups.

geted regulation between them. This work investigated whether miR-195 played a role in mediating MAP2K1 expression and affecting proliferation, apoptosis and ADM resistance of prostate cancer cells. Dual-Luciferase gene reporter assay showed that the transfection of miR-124 mimic remarkably decreased relative Luciferase activity of HEK293T cells transfected with pLUC-MAP2K1-WT plasmid, suggesting the targeted regulation between miR-195 and MAP2K1 mRNA. CCK-8 assay established that drug-resistant prostate cancer cell line DU145/ADM had remarkably higher IC_{50} values than DU145 cells. Similarly, PC-3/ADM cells also had remarkably higher IC_{50} values than parental PC-3 cells, suggesting the acquisition of AMD resistance in DU145/ADM and PC-3/ADM cells. Flow cytometry for apoptosis also revealed remarkably lower sensitivity of ADM-induced cell apoptosis in DU145/ADM and PC-3/ADM cells, producing apoptotic resistance. Both proliferation and apoptosis assay showed the successful generation of prostate cancer cell lines with ADM drug resistance that can satisfy the requirement of further experiments. Compared to normal prostate [illegible] epithelial cell line RWPE-1, prostate ca[illegible] lines DU145 and PC-3 showed significa[illegible] decreased miR-195 expression, which was f[illegible]r down-regulated in drug-resistant cell [illegible] DU145/ADM and PC-3/ADM. Prostate can[illegible] cell line also showed si[illegible] highe[illegible] MAP2K1 mRNA and pro[illegible] exp[illegible]on than normal prostate gland [illegible]ithelial [illegible]ls, and drug-resistant cells had [illegible] highe[illegible] expression than pare[illegible]al n[illegible] [illegible]. The results showed [illegible]at the d[illegible]gulation of miR-195 played i[illegible]ortant roles i[illegible]ntiating MAP2K1 expr[illegible]d its participa[illegible]n in the regulation of [illegible]state [illegible]r cell drug resistance in addition to prostate c[illegible]. In a correlation study bet[illegible]en miR-195 and p[illegible]te cancer, Guo et al[34] f[illegible]d significantly decre[illegible]sed miR-195 express[illegible] in tum[illegible] tissues, and its potency as the pre[illegible] fac[illegible]or patient survival and prognosis. Zh[illegible][3] showe[illegible]hat, compared to low-grade pro[illegible] cancer [illegible]ssues, high-grade cancer [illegible] pres[illegible]iR-195 down-regulation, wh[illegible] down-reg[illegible]on is related to worse surviva[illegible]d prognosis. Cai et al[14] demonstrated that, co[illegible]mal prostate epithelial cell PrEC, [illegible]oma cells PC-3, LNCaP and DU145 [illegible]howed remarkably decreased miR-195 exp[illegible] plus miR-195 down-regulation in prostate [illegible]cer patients and the correlation between miR-195 low expression and unfavorable [illegible] or prognosis of patients ($p = 0.009$). Th[illegible] indicated the participation of miR-1[illegible] down-re[illegible]ulation in the pathogenesis of pr[illegible]te cancer, as our reuslts also showed. Curre[illegible] few studies have been performed regarding [illegible]ulation of prostate cancer cell's drug [illegible]sistanc[illegible]R-195. Ma et al[12] found that, co[illegible]red to drug [illegible] cell DU15, docetaxel ([illegible]C) resistant cell D[illegible] DOC had remarkabl[illegible]creased [illegible]iR-195 expression, indicating tha[illegible] down-re[illegible]lation was a regulator[illegible] facto[illegible]rug res[illegible]nce of prostate canc[illegible] cells, fu[illegible]up[illegible]ing our study. To f[illegible]r investigate [illegible]r miR-195 played a [illegible]diating MAP2[illegible]1 expression and dru[illegible]esista[illegible] prostate cancer cells, this study over-expresse[illegible]R-195 in established pro[illegible]ncer cells w[illegible]g resistance to obs[illegible]ological effects. The results showed that [illegible] transfection of miR-195 mimic remarkably [illegible]creased the [illegible]ession of the MAP2K1 pro[illegible] in DU145/A[illegible]M and PC-3/ADM cells, and s[illegible]sed ERK[illegible]MPK pathway activity, leading [illegible]ly higher apoptosis of those drug-resis[illegible] cells that originally have stable [illegible]wth in ADM-containing medium; in addition [illegible]ssed cell proliferation and malignant [illegible]olog[illegible]l properties. Cai et al[14] showed that the up-regulation of miR-195 can suppress migration or invasion potency of prostate cancer cells LNCaP and DU145 *via* targeted inhibition RPS6KB1 expression to facilitate cell apoptosis, and can inhibit growth or tumorigenesis of tumor cells in BALB/c nude mice to suppress cell invasion or infiltration. Guo et al[34] found that the over-expression of miR-195 targets and inhibits BCOX1 gene expression, suppress proliferation, migration or invasion of *in vitro* cultured prostate cancer cells PC-3 or LNCaP, thus weakening *in vivo* growth, tumorigenesis and distal metastasis potency of those cells. Wu et al[35] found that the over-expression of miR-195 could target and regulate Fra-1 to weaken migration or invasion potency of prostate cancer cells PC-3 and DU145. It has been found that the over-expression of miR-195 could target FGF2 to suppress epithelial-mesenchymal transition (EMT) process of PC-3 or DU145 cells, thus weakening migration or invasion of cells. These studies all confirmed the role of miR-195 in weakening malignant biological properties of prostate cancer cells, supporting our results. In a study for the miR-195 regulation of prostate cancer cell's drug resistance, Ma et al[12] found that miR-195

RETRACTED

RETRACTED

played a role in targeted regulation of CLU, and over-expression of miR-195 can target and inhibit CLU expression to facilitate apoptosis of drug-resistant cells DU145/DOC and inhibit its clonal formation ability, thus suppressing DOC resistance of cells. In contrast to this study, we identified the role of miR-195 in targeted inhibition of the MAP2K1 expression, suppression of ERK/MAPK signal pathway transduction and weakening of prostate cancer cell's drug resistance, all of which have not been previously documented. However, this work has certain limitations as whether the regulation of MAP2K1 expression by miR-195 is correlated with drug resistance of prostate cancer patients is still unclear, which requires further assays for describing differential expression of miR-195 and MAP2K1 in tumor tissues from chemotherapy-sensitive and resistant patients.

Conclusions

We showed that the down-regulation of miR-195 was correlated with ADM resistance of [illegible]tate cancer cells. The up-regulation of [illegible] can weaken proliferation potency of d[illegible]resistant prostate cancer cells *via* targeted [illegible]tion on MAP2K1 expression and weakenin[illegible] the ERK/AMPK signal pathway to facilitate c[illegible] apoptosis and suppress ADM [illegible]

Conflict of Interest

The Authors declare that they ha[illegible]

Acknowledgen[illegible]

This work was s[illegible]orte[illegible] Fujian Provincial Natural Science Foundat[illegible] of Chin[illegible]018J01282).

References

1) [illegible] W[illegible]XX, Fu H[illegible] Tang YC, Meng BQ, Chen C[illegible]agnostic[illegible] of PSA combined miR-155 [illegible]n in p[illegible]ate cancer. Eur Rev Med [illegible]harma[illegible] 22: 1615-1621.
2) [illegible]operberg [illegible]an JM. Epidemiology of pros[illegible] cancer. W[illegible]d J Urol 2017; 35: 849.
3) [illegible] Kyprianou N. Profiling prostate cancer [illegible]resistance. Int J Mol Sci 2018; 19: E904.
4) [illegible]lig Z. Molecular mechanisms of enzalutamide [illegible]tance in prostate cancer. Curr Mol Biol Rep [illegible]; 3: 230-235.
5) Li L, Wang J, Zhang Y, Ma L, Weng W, Qiao Y, Xiao W, Wang H, Yu W, Pan Q. MEK1 pr[illegible] and their interaction is critical f[illegible] sis in liver cancer. FEBS letters [illegible]; 587: 392[illegible] 3927.
6) Penzvalto Z, Lanczky A, Len[illegible] Meggyeshazi N, Krenacs T, Szoboszlai N, Denkert [illegible], Gyorffy B. MEK1 is associated wi[illegible] carbop[illegible]stance and is a prognostic bio[illegible]er in epithe[illegible] cancer. BMC Cancer [illegible]4; 14: 837.
7) Gysin S, Paquette J[illegible]Mahon M. Analysis of [illegible]NA profiles after[illegible]2 inhi[illegible]n in human pancreatic cancer ce[illegible]eals path[illegible]s involved in dr[illegible]ensit[illegible] Cance[illegible]s 2012; 10: 1607-1[illegible]
8) Kato T, [illegible]Y, Nakane K, Mi[illegible] Terazawa R, Ehara [illegible] Y, Kojima T, [illegible]awa Y, Deguchi T. C[illegible]/CC[illegible]action promotes invasion of taxane-resistant [illegible]rostate cancer cells by increasing secretion [illegible]s 2/9 and by activating [illegible]nd Rac signalin[illegible]tokine 2013; 64: 251-257.
9) Lescarbeau RM, Seib FP, Prewitz M, Werner C, Kaplan DL. In v[illegible] model of metastasis to bone marrow mediates [illegible]state cancer castration resistant [illegible]owth throu[illegible]aracrine and extracellular matrix [illegible] Pl[illegible]ne 2012; 7: e40372.
10) A[illegible], Liu C, Lou W, Lombard AP, Evans CP, Gao AC. MicroRNA-181a promotes docetaxel resistance in prostate cancer cells. Prostate 2017; [illegible]0-1028.
11) L[illegible] Mahato RI. MicroRNAs and drug resistance in prostate cancers. Mol Pharm 2014; 11: 2539-2552.
12) Ma X, Zou L, Li X, Chen Z, Lin Q, Wu X. MicroRNA-195 regulates docetaxel resistance by targeting clusterin in prostate cancer. Biomed Pharmacother 2018; 99: 445-450.
13) Zhang X, Tao T, Liu C, Guan H, Huang Y, Xu B, Chen M. Downregulation of miR-195 promotes prostate cancer progression by targeting HMGA1. Oncol Rep 2016; 36: 376-382.
14) Cai C, Chen QB, Han ZD, Zhang YQ, He HC, Chen JH, Chen YR, Yang SB, Wu YD, Zeng YR. miR-195 inhibits tumor progression by targeting RPS6KB1 in human prostate cancer. Clin Cancer Res 2015; 21: 4922-4934.
15) Arcangeli S, Pinzi V, Arcangeli G. Epidemiology of prostate cancer and treatment remarks. World J Radiol 2012; 4: 241-246.
16) Brawley OW. Prostate cancer epidemiology in the United States. World J Urol 2012; 30: 195-200.
17) Fenner A. Prostate cancer: ERSPC calculator recalibrated for China. Nat Rev Urol 2017; 14: 66.
18) Ren SC, Chen R, Sun YH. Prostate cancer research in China. Asian J Androl 2013; 15: 350-353.
19) Wang DW, Wang YQ, Shu HS. MiR-16 inhibits pituitary adenoma cell proliferation via the suppression of ERK/MAPK signal pathway. Eur Rev Med Pharmacol Sci 2018; 22: 1241-1248.

20) Li B, Liu YH, Sun AG, Huan LC, Li HD, Liu DM. MiR-130b functions as a tumor promoter in glioma via regulation of ERK/MAPK pathway. Eur Rev Med Pharmacol Sci 2017; 21: 2840-2846.

21) Buchegger K, Silva R, Lopez J, Ili C, Araya JC, Leal P, Brebi P, Riquelme I, Roa JC. The ERK/MAPK pathway is overexpressed and activated in gallbladder cancer. Pathol Res Pract 2017; 213: 476-482.

22) Zhang G, Cheng Y, Zhang Q, Li X, Zhou J, Wang J, Wei L. ATXLPA axis facilitates estrogeninduced endometrial cancer cell proliferation via MAPK/ERK signaling pathway. Mol Med Rep 2018; 17: 4245-4252.

23) Liao T, Wen D, Ma B, Hu JQ, Qu N, Shi RL, Liu L, Guan Q, Li DS, Ji QH. Yes-associated protein 1 promotes papillary thyroid cancer cell proliferation by activating the ERK/MAPK signaling pathway. Oncotarget 2017; 8: 11719-11728.

24) Lu Y, Li Y, Xiang M, Zhou J, Chen J. Khat promotes human breast cancer MDA-MB-231 cell apoptosis via mitochondria and MAPK-associated pathways. Oncol Lett 2017; 14: 3947-3952.

25) Yang XL, Liu KY, Lin FJ, Shi HM, Ou ZL. CCL28 promotes breast cancer growth and metastasis through MAPK-mediated cellular anti-apoptosis and pro-metastasis. Oncol Rep 2017; 38: 1393-1401.

26) Zhuang ST, Cai Y, Gao HC, Qiu JF, Zeng J [illegible] WJ. Study on the function and mechanism [illegible] non-coding RNA DMTF1v4 in the occu[illegible]ce of colon cancer. Eur Rev Med Pharmacol S[illegible]18; 22: 3779-3788.

27) McGivern N, El-Helali A, Mullan P, McNeis[illegible] Paul Harkin D, Kennedy RD, McCa[illegible] N. Activat[illegible] of MAPK signalling results in [illegible]ce to sara[illegible] catinib (AZD0530) in ovari[illegible] [illegible]cotarget 2018; 9: 4722-4736.

28) Hew KE, Miller PC, El-Ashry D, Sun J, Besser AH, Ince TA, Gu M, Wei Z, Zhang G, Braffo[illegible] Lu Y, Mills GB, Slingerland JM, Si[illegible] activation predicts poor outcome [illegible] the MEK in[illegible] hibitor, selumetinib, reverses a[illegible]strogen resistance in ER-positive high-gr[illegible]erous ovarian cancer. Clin Cancer Res 2016; [illegible]5-947.

29) Zhao L. Hirudin inhibits cell gro[illegible] ERK/MAPK signaling in hu[illegible] glioma. Int [illegible] Ex[illegible] Med 2015; 8: 20983-[illegible]87.

30) Chiba T, Soeno Y, S[illegible]o Y, Sudo [illegible] Yagishita H, [illegible]ya Y, Kawashiri S, O[illegible] Imai K[illegible]LT1 Inhibition of oral carcinoma ce[illegible]nd ERK/M[illegible]K activation. J De[illegible] Res 2[illegible] 446-45[illegible]

31) Yu X, Zhan[illegible] Cavazos D, [illegible] Du L, Pertsemlidis A[illegible]-195 targets cy[illegible] and survivin to mo[illegible] tumorigenesis [illegible]non-small cell lung [illegible]cer. [illegible]ath Dis 2018; 9: 193.

32) Feng C, Zhang L, [illegible] X, Zhan L, Lou Y, Wang Y, [illegible] Zhang Y. GDP[illegible]rget of miR-195-5p, is [illegible]ated with metasta[illegible] and chemoresistance in colorectal cancer. Biomed Pharmacother 2018; 101: 945-952.

[illegible] Zhou B, Sun [illegible] X, Zhan H, Zou H, Feng Y, Qiu F, Zhang S, Wu L [illegible]ang B. MicroRNA-195 suppress[illegible] the progre[illegible]on of pancreatic cancer by target[illegible] [illegible] Physiol Biochem 2017; 44: 1867-18[illegible]

34) Guo J, Wang M, Liu X. MicroRNA-195 suppresses [illegible]mor cell proliferation and metastasis by direct[illegible] [illegible]eting BCOX1 in prostate carcinoma. J Exp Cli[illegible] Cancer Res 2015; 34: 91.

35) Wu J, Ji A, Wang X, Zhu Y, Yu Y, Lin Y, Liu Y, Li S, Liang Z, Xu X, Zheng X, Xie L. MicroRNA-195-5p, a new regulator of Fra-1, suppresses the migration and invasion of prostate cancer cells. J Transl Med 2015; 13: 289.

RETRACTED