European Review for Medical and Pharmacological Sciences 2019; 23: 8795-8805

RETRACTED

MiR-605-3p inhibits malignant progression of prostate cancer by up-regulating EZH2

M.-Z. PAN[1], Y.-L. SONG[2], F. GAO[3]

[1]Department of Urology Surgery, The Seventh People's Hospital of Jinan, [illegible]n, China
[2]Department of Otorhinolaryngological, The Seventh People's Hospital [illegible]nan, Ji[illegible]n, China
[3]Department of Neurosurgery, The Seventh People's Hospital of Jinan, [illegible] Chi[illegible]

Abstract. – **OBJECTIVE:** This study aimed to investigate the effect of microRNA-605-3p (miR-605-3p) on cell proliferation, invasion and migration in prostate cancer cells, as well as its effects on the Enhancer Zeste Homolog 2 (EZH2) expression and the potential regulatory mechanisms.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was applied to detect the miR-605-3p expression in 52 pairs of Prostate Cancer (PCa) tissues and normal tissues, and to analyze the relatio[illegible]ship between miR-605-3p expression and PCa [illegible] logical parameters. MiR-605-3p mimi[illegible] were transfected into PCa cell line PC-3 and D[illegible] to achieve miR-605-3p overexpression. Then [illegible]ll Counting Kit-8 (CCK-8), cell colony forma[illegible] assay and transwell invasion and migration [illegible] say were performed to analyze [illegible]05-3p's e[illegible] fect on the biological functi[illegible] [illegible]ate cancer cells. Finally, the pote[illegible]al mec[illegible]isms of downstream genes were [illegible]plored th[illegible]ugh bioinformatics analysis and [illegible]ery e[illegible]

RESULTS: We foun[illegible] that [illegible]3p expression in PCa was [illegible]kedly l[illegible]han that in normal tissues. P[illegible]ents with l[illegible]R-605-3p expression had [illegible] tumor stage, [illegible]r incidence of lymph[illegible] [illegible]astasis and distant metastasis. In v[illegible] analy[illegible]owed that miR-605-3p overexp[illegible]ssion leads t[illegible]nificant decrease in cell pr[illegible]eration, invasion [illegible] migration ability. Sub[illegible]quently, it was verifie[illegible] in cell line and tissu[illegible]at EZH2 expression was remarkably incre[illegible] in PCa [illegible]hich was negatively correlated with [illegible]05[illegible] expres[illegible]n. In addition, the recovery [illegible]ent fou[illegible]hat EZH2 can counter-[illegible] the ro[illegible]R-6[illegible]3p in PCa, together they [illegible]d the [illegible] progression of PCa.

[illegible]CLUSION[illegible]: [illegible]iR-605-3p was significantly ass[illegible]ted with PCa stage, lymph node metasta[illegible] [illegible] metastasis and the poor progn[illegible], it can inhibit the malignant pro[illegible]ssion of PCa. In addition, the study showed [illegible]iR-605-3p may inhibit the proliferation, inva[illegible]nd migration ability of PCa by regulating E[illegible]2.

Key Word[illegible]

MiR-60[illegible]p, EZ[illegible]state cancer, Malignant progression.

Introduction

[illegible]rostate Canc[illegible]PCa) is one of the most common [illegible]alignant t[illegible]ors in the world, which is also an [illegible]se of cancer-related deaths in western [illegible]ries. In recent years, the incidence [illegible] PCa in China has increased rapidly year by [illegible]er the introduction of prostate-specif-[illegible] (PSA) screening, the detection rate of prostate cancer peaked in the early 1990s[4,5]. At present, approximately 85% of men with newly diagnosed prostate cancer are confined to early tumors[6]. Although the popularity of conventional PSA screening has increased the rate of early detection of prostate cancer, its benefits are still controversial, mainly because there is no consensus on whether PSA can effectively reduce prostate cancer mortality[7-9]. Due to the characteristics of tumor heterogeneity in prostate cancer, exploring new molecular markers for prostate cancer diagnosis and prognosis stratification, as well as new molecular therapeutic targets have become the main task for current researchers. Accelerating the diagnosis, prognosis and therapeutic effects of prostate cancer have important clinical implications for opening up a new direction in precision medicine[9,10]. In addition to transcriptional factors that have shown clinical utility in a variety of clinical settings, researchers[11,12] are increasingly interested in the potential effects of microRNAs (miRNAs) as biomarkers for prostate cancer and targeted therapies.

MicroRNAs (miRNAs) are non-coding single-stranded RNA molecules of approximately 18-22 nucleotides in length that serve primar-

Corresponding Author: Meizhou Pan, BM; e-mail: 13953190521@163.com

RETRACTED

ily as post-transcriptional gene regulators[12,13]. MiRNAs could bind to the 3' untranslated region (UTR) of the target mRNA, or incomplete bind to the 5'-UTR or coding sequence, thereby altering their stability[14]. Thus, miRNAs can directly target mRNA degradation in a fully complementary manner, or induce translational inhibition by incomplete binding[15]. Some individual miRNAs have been shown to be tumor suppressor genes or oncogenes, depending on their regulated downstream targets, which make them more potentially useful[16]. MiR-605-3p is a non-coding short-stranded RNA expressed on human chromosome 10. It has been shown to be abnormally expressed in many tumor tissues, indicating that the interference of miR-605-3p is closely related to tumors. MiR-605-3p performs different functions for different tumors, and some scholars[17] have reported that it can inhibit bladder cancer. Therefore, the focus and difficulty in the miRNA research work have been to discover and clarify the target genes regulated by miRNAs and the signaling pathways involved.

Enhancer of zeste homolog 2 (EZH2) is a [illegible] ber of the PeG (Polycomb group genes [illegible] and is closely related to embryonic devel[illegible]ent, cell differentiation, self-renewal of cancer [illegible] cells and the pathogenesis of a variety of m[illegible] nancies[18,19]. It is worth noting that EZH2 is loc[illegible] ed on human chromosome 7q3[illegible] [illegible]mparativ[illegible] genomic hybridization (CGH [illegible] confir[illegible] at there may be genes associated w[illegible] prostate [illegible]cer metastasis in this chromoso[illegible]ion[20]. [illegible] a catalytic subunit of PCR2 [illegible] repre[illegible] complex 2), which e[illegible]des a pr[illegible] with a highly conserved SET [illegible]ain that can [illegible]ited by trimethylating [illegible] histone H3 at [illegible]tion 27 lysine (H3K2[illegible] A se[illegible] effector proteins, such as DNA methyltransferas[illegible]alyze DNA methylation to [illegible]vent transcripti[illegible]ors from binding to t[illegible] promoter region and [illegible]imately achieve gene [illegible]ncing[21,22]. Researchers[23,24] have shown that the [illegible]sion [illegible]order of EZH2 is closely in association [illegible] occurre[illegible] and development of a variety of [illegible] and [illegible] high expression can pro[illegible] [illegible]he inva[illegible] metastasis of cancer cells.

[illegible]d on the [illegible]ve series of results, we further [illegible]plored the role of miR-605-3p and EZH2 in [illegible]r and described the possible role [illegible] and EZH2 in the development of [illegible]tate cancer separately. Besides, we explained th[illegible]cular regulation mechanism of miR-605-3p o[illegible]e action of EZH2, which may bring new ideas for the diagnosis and treatment of prostate cancer.

Patients and Me[illegible]ds

Patients and PCa Sam[illegible]es

Tumor tissues and [illegible]acancerous [illegible] of 52 patients aged 4[illegible]8 years who re[illegible] radical prostatecto[illegible] were co[illegible]cted. None of the patients underw[illegible]di[illegible]rapy or [illegible]hemotherapy before s[illegible]gery. [illegible]ogical t[illegible]ng and staging criter[illegible] for prosta[illegible]ce[illegible]ere performed in a[illegible]dance with in[illegible]nal cancer against c[illegible]CC) prostate [illegible]ncer staging criteria. [illegible]tients [illegible]heir families in this study have been fully in[illegible], and informed consent [illegible]een signed a[illegible]proved by the Ethi[illegible]ight Committee.

[illegible]ll Lines an[illegible]eagents

[illegible]our human-[illegible]ved PCa cell lines (PC-3, 22[illegible] Lncap, [illegible]-145) and one normal human pros[illegible]mortalized cell line (WPMY-1) were purchased from the American Type Culture [illegible]llection (ATCC; Manassas, VA, USA). F-12k [illegible]640 medium as well as fetal bovine se[illegible]m ([illegible]S; Gibco, Grand Island, NY, USA) used for cell culture were purchased from Life Technologies (Gaithersburg, MD, USA). Cells were cultured in a 37°C, 5% CO_2 incubator.

Cell Transfection

The negative control (NC) and the miR-605-3p overexpression sequence (miR-605-3p mimics) were constructed by the Jima Company (Shanghai, China). Cells were plated in 6-well plates and grown to a cell density of 70%, then transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the instructions. Cells were collected for quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) analysis or other functional analysis after 48 hours.

Cell Proliferation Assays

The cells after 48 h of transfection were harvested and plated into 96-well plates at 2000 cells per well. After culturing for 24 h, 48 h, 72 h and 96 h, the cells were added with Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) reagent and incubated for 2 hours. Then, the optical density (OD) value of each well was measured using the microplate reader at 490 nm.

RETRACTED

Colony Formation Assay

After the cell density reached 70-80%, a single cell suspension was prepared by digesting with 0.25% trypsin. Subsequently, the cell suspension was repeatedly blown with a pipette tip to more than 95% of the cells to form a single cell, and the number of viable cells was counted by the cell counting plate after trypan blue staining. 100 cells were plated in a 6-well plate with 2 ml of complete medium. Three parallel samples were set for each concentration, and the medium was changed every other day. Cells were cultured in a 37°C, 5% CO 2 incubator for 7 days to calculate the colony formation rate.

Transwell Cell Migration and Invasion Assays

After transfection for 48 hours, the cells were resuspended in a serum-free medium. After cell counting, the diluted cell density was adjusted to 2.0×10^5/mL, and the transwell chamber containing Matrigel and no Matrigel was placed in a 24-well plate. 200 µL of the cell suspension was added to the upper chamber, and 500 µL of a medium was added to the lower chamber. Af[illegible] hours, the chamber was removed, fixed [illegible] paraformaldehyde for 30 minutes, stain[illegible] with the crystal violet for 15 minutes and then w[illegible]d with Phosphate-Buffered Saline (PBS; [illegible]co, Grand Island, NY, USA). The inner surfa[illegible] of the basement membrane of [illegible]mber wa[illegible] carefully cleaned to remove [illegible] inne[illegible]er cells. The perforated cells stain[illegible]n the ou[illegible] layer of the basement membrane [illegible] chamb[illegible] served under the micr[illegible]scope[illegible] were randomly sele[illegible] for cou[illegible]

Quantitative [illegible]me-Polymera[illegible] Chain Reac[illegible]n

TRIzol (Invitrogen, C[illegible]d, CA, USA) was used to e[illegible]act total RNA. [illegible]itially extracted RN[illegible] was treated with DN[illegible]se I to remove geno[illegible] DNA [illegible]nd repurify the RNA. RNA rev[illegible]trans[illegible]tion was performed according to [illegible]he Scri[illegible] Reverse Transcription Kit (Tak[illegible] Otsu, [illegible]iga, Japan) instructions [illegible] Real [illegible]ymerase Chain Reaction wa[illegible]rformed [illegible]ording to the SYBR® Premix [illegible]aKaRa, Otsu, Shiga, Japan) kit instructio[illegible] StepOne Plus Real Time-PCR S[illegible]em (Ap[illegible]ed Biosystems, Foster City, CA, [illegible]). Primers were listed below. MiR-605-3p: f: 5[illegible]AGGCACUAUGAGAUUUAGA-3'; U6: f: [illegible]CGCAAGGATGACACGCAAATTC-3''; EZH2 : f:5'-TCCGCGCCCTTGCCCAGA[illegible]C-3', r: 5'-GCCGCACGAACAGCCCCA[illegible] β-actin: f: 5'-CCTGGCACCCAGC[illegible]AAT-3', [illegible] 5'-GCTGATCCACATCTGCTG[illegible]A-3'.

Each sample was subjecte[illegible] a three-hole repeated experiment and the PC[illegible] repeated twice. Bio-Rad PCR instr[illegible]ent wa[illegible] to analyze and process the da[illegible] with softwa[illegible] (Hercules, CA, USA), [illegible]e β-actin and U6 g[illegible] were used as interna[illegible]ameter[illegible]

Western Blot

The transfe[illegible] cells we[illegible]d u[illegible]g cell lysis buffer an[illegible]ntrifuged at 12[illegible]or 20 minutes at 4°[illegible]rotein concen[illegible]ion was calculated [illegible] Prote[illegible]ay Kit (Pierce, Waltham, MA, USA). Primary [illegible]ody (Anti-EZH2) was pur[illegible] from Santa C[illegible] Biotechnology (Sant[illegible]ruz, CA, USA), whi[illegible] horseradish peroxi[illegible]se-labeled secondary antibody was purchased [illegible]m GenScript [illegible]cataway, NJ, USA).

[illegible]odium do[illegible]yl sulphate-polyacrylamide g[illegible]ectropho[illegible]s (SDS-PAGE) electrophoresis [illegible]ned to separate proteins, then the prote[illegible] were transferred to polyvinylidene [illegible]fluoride (PVDF) membrane (Millipore, Bill[illegible], USA). After blocking overnight in [illegible]ilk, the membranes were incubated with the primary antibodies and secondary antibodies separately. Proteins bands were detected by electrochemiluminescence (ECL; Thermo Fisher Scientific, Waltham, MA, USA), while the image was semi-quantitatively analyzed by alpha SP image analysis software.

Statistics Analysis

Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) 22. 0 software (IBM, Armonk, NY, USA). The χ^2 test and the exact probability Fisher test as well as COX regression analysis were performed respectively for the univariate analysis or the multivariate analysis. The Kaplan-Meier method was used for patient survival analysis. Data were expressed as mean ± standard deviation ($\bar{x}\pm s$), and $p<0.05$ was considered statistically significant.

Results

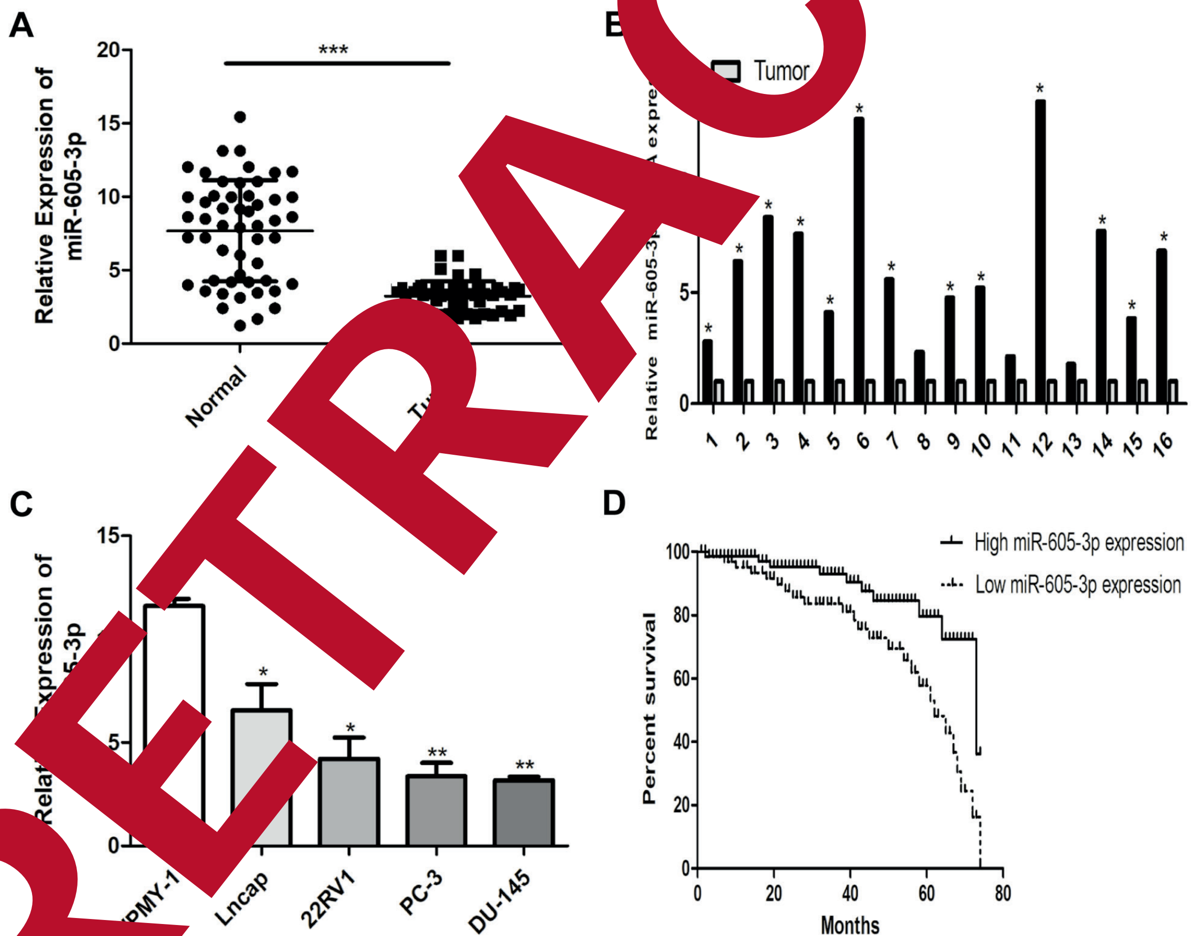
MiR-605-3p Was Lowly Expressed in PCa

MiR-605-3p expressions in PCa tissues as well as cell lines were verified by qRT-PCR, which revealed that miR-605-3p was markedly decreased

in PCa tissues than that of the adjacent tumor-free tissues (Figure 1A and 1B). In addition, miR-605-3p was significantly decreased in PCa cell lines, especially in PC-3 and DU145 cells, where the difference in expression levels was the most marked, so we chose these two strains for further analysis (Figure 1B).

MiR-605-3p Expression Was Associated with Clinical Stage, Lymph Node and Distance Metastasis in PCa Patients

According to the level of miR-605-3p in 52 pairs of PCa tissues and adjacent tissues, patients were divided into 2 different groups: the high miR-605-3p expression group and low miR-605-3p expression group. The relationship between miR-605-3p expression and the age, sex and clinical stage, lymph node metastasis and distant metastasis of PCa patients were an[illegible] ble I showed that low expression o[illegible]iR-605-[illegible] was positively correlated with PC[illegible]linical stage, lymph node metastasis and dist[illegible]etastasis, but not with age and gender. These [illegible] suggested that miR-605-3p may be a [illegible]w biol[illegible]indicator for predicting the m[illegible]gnant prog[illegible] PCa. In addition, to e[illegible]re the relationsh[illegible]tween miR-605-3p e[illegible]ession a[illegible] the prognosis of PCa patients, we [illegible]ted [illegible]evant fo[illegible]ow-up data. Kaplan-Me[illegible]r sur[illegible]rves rev[illegible]ed that miR-605-3p l[illegible] expression [illegible]tro[illegible]y associated with th[illegible]r prognosis o[illegible]igure 1D), demonstra[illegible]g [illegible]miR-605-3p ex[illegible]ession was in correlat[illegible] with [illegible]l stage, lymph node and distance metastasis i[illegible] patients.



[illegible]re 1. MiR-605-3p is under-expressed in prostate cancer tissues and cell lines. **A-B,** qRT-PCR detection of differential [illegible]ion of miR-605-3p in prostate cancer tissues and adjacent non-tumor tissues. **C,** qRT-PCR detection of miR-605-3p ex[illegible] levels in prostate cancer cell lines. **D,** Kaplan-Meier survival curve of lung cancer patients based on FOXC2-AS1 expres[illegible]n. Data are expressed as mean ± SD, *p<0.05, **p<0.01, ***p<0.001.

RETRACTED

Table I. Association of miR-605-3p expression with clinicopathologic characteristics of prostate cancer.

Parameters expression	Number of cases	miR-605-3p		p-value
		High (%)	Low (%)	
Age (years)				[illegible]
<60	20	12	8	
≥60	32	19	[illegible]	
Gender				[illegible]
Male	25	17	8	
Female	27	14	13	
T stage				[illegible]
T1-T2	31	22	[illegible]	
T3-T4	21	9	[illegible]	
Lymph node metastasis				[illegible]
No	33	25	8	
Yes	19	6	13	
Distance metastasis				0.033
No	38	26	[illegible]	
Yes	14	[illegible]	[illegible]	

MiR-605-3p Overexpression Attenuated Cell Proliferation, Migration and Invasion

To better understand the role of miR-605[illegible] in PCa, we constructed the miR-605-3p mi[illegible] pression model and verified miR-605-3p [illegible]sion by qRT-PCR (Figure 2A). It was foun[illegible]t the proliferation rate of cells in the miR-60[illegible] overexpression group was markedly decreas[illegible] than that in the NC group by CC[illegible] prolifera[illegible]tion assay and cell colony for[illegible]tion a[illegible] (Figure 2B and 2C). In addition, w[illegible]pplied th[illegible]ranswell migration assay to inves[illegible] the ef[illegible] 605-3p on PCa cell m[illegible]gratio[illegible] [illegible] results showed that t[illegible] number [illegible]membrane PCa cells in the [illegible]swell chamb[illegible] miR-605-3p overexp[illegible]as remarkab[illegible]educed, suggesting th[illegible]e mi[illegible]n invasive ability was inhibited (Figure 2D).

EZH2 [illegible]s Highly Expresse[illegible] in PCa

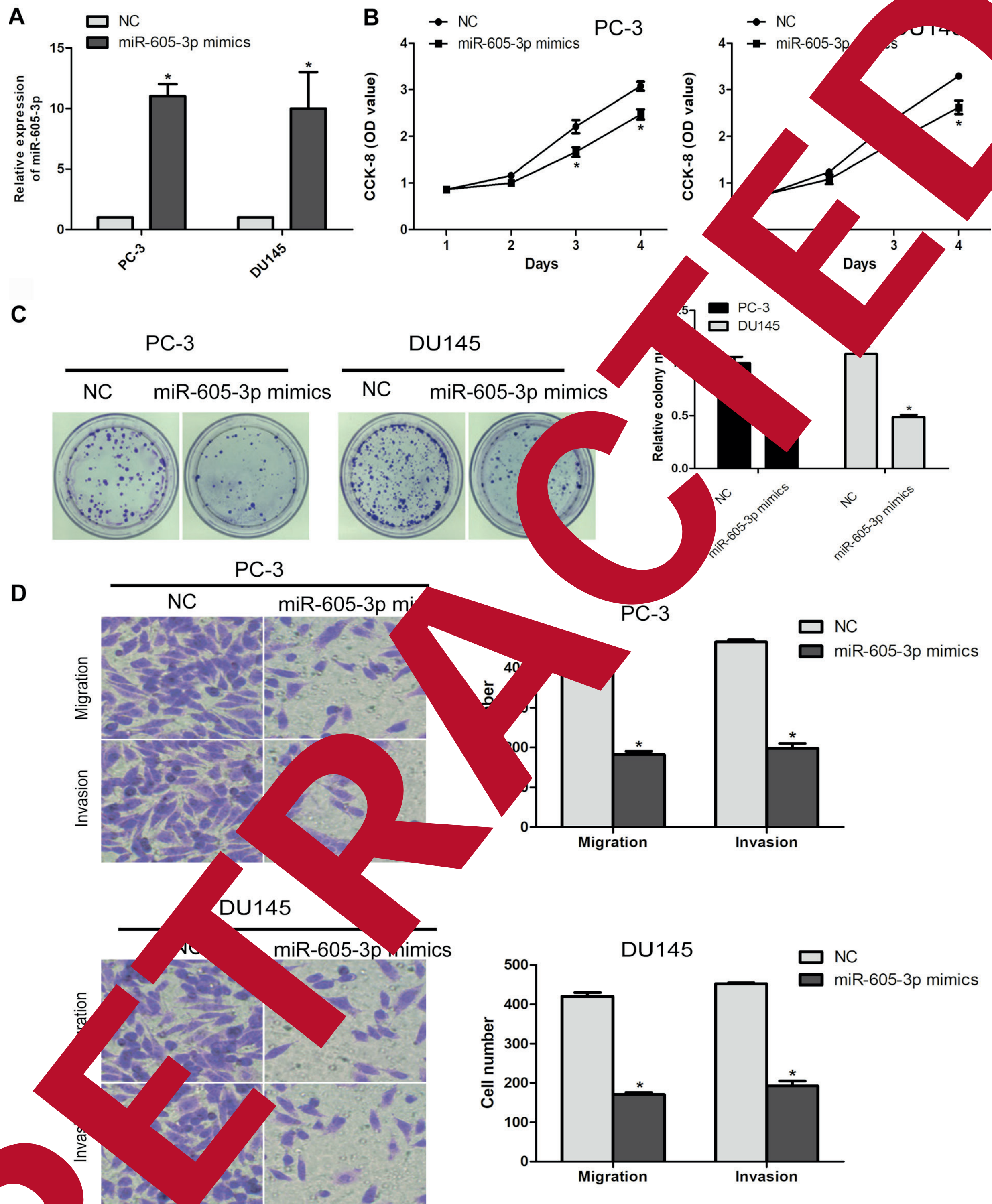
E[illegible] expres[illegible]on in PCa tissue and cell line wa[illegible]ed b[illegible]RT-PCR. The results displayed that t[illegible]evel in [illegible] tissue was markedly increased [illegible]red w[illegible] the adjacent tumor-free [illegible] (Figur[illegible]ddition, EZH2 expression in [illegible]cells was[illegible]nificantly higher when compare[illegible]ith that in the WPMY-1 cell line (Figure 3B)[illegible] we observed a negative correla[illegible] [illegible]betwe[illegible]miR-605-3p expression and EZH2 [illegible]ssion by qRT-PCR in prostate cancer tissues a[illegible] lines (Figure 3C and 3D), indicating that miR-[illegible]-3p might target EZH2 in PCa.

E[illegible] Modula[illegible]d MiR-605-3p Expression in [illegible]

To fu[illegible] explore the way in which miR-605-[illegible] inhibited the malignant progression of prostate [illegible] overexpressed both EZH2 and miR-[illegible]5-3p [illegible] the cell lines of PCa to explore their role in PCa. The transfection efficiency of EZH2 was examined by qRT-PCR and Western blot (Figure 4A and 4B). Further experiments including cell colony formation assay and transwell migration invasion assay showed that EZH2 can counteract the proliferation, invasion and metastasis of miR-605-3p in PCa cells (Figure 4C and 4D), suggesting that EZH2 modulated miR-605-3p expression in human PCa cells

Discussion

Prostate cancer is one of the most common male malignancies, which has caused widespread concern in the world today, yet there is no clear evidence in the epidemiology of its exact predisposing factors[1-3]. At present, the initial cell population of malignant tumors is called cancer stem cells (CSCs). In prostate cancer research, prostate cancer stem cells are still a hot spot in the world[25]. Genetic polymorphism also plays a very important part. Although there have been a lot of researches on the mechanism of prostate cancer, the specific mechanism remains unclear. This may be because the malignant tumors are often accompa-



RETRACTED

Fig... miR-605-3p affects prostate cancer cell proliferation, invasion and migration. **A,** qRT-PCR validates the trans-fe... of miR-605-3p after transfection of NC and miR-605-3p mimics in PC-3 and DU145 cell lines. **B,** CCK-8 ...y is performed to detect the effect of miR-605-3p on prostate cancer cell proliferation in PC-3 and DU145 cell line. **C,** Cell ... formation assay is performed to detect the effect of miR-605-3p on cell proliferation in PC-3 and DU145 cell lines. **D,** Tr... migration and invasion assay is performed to detect the effect of miR-605-3p on invasion and migration in prostate cance... in PC-3 and DU145 (magnification: 40×). Data are shown as mean ± SD, $*p<0.05$.

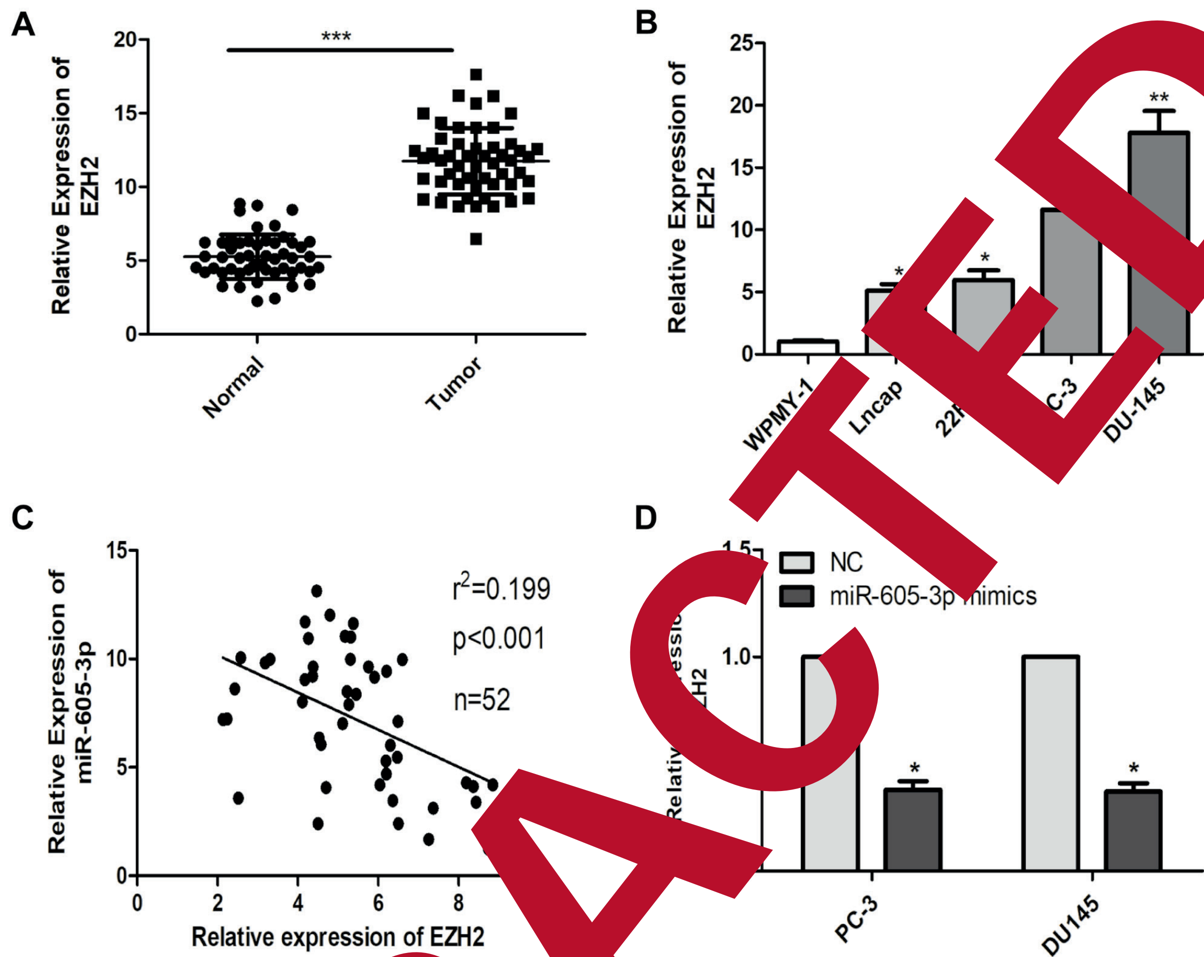
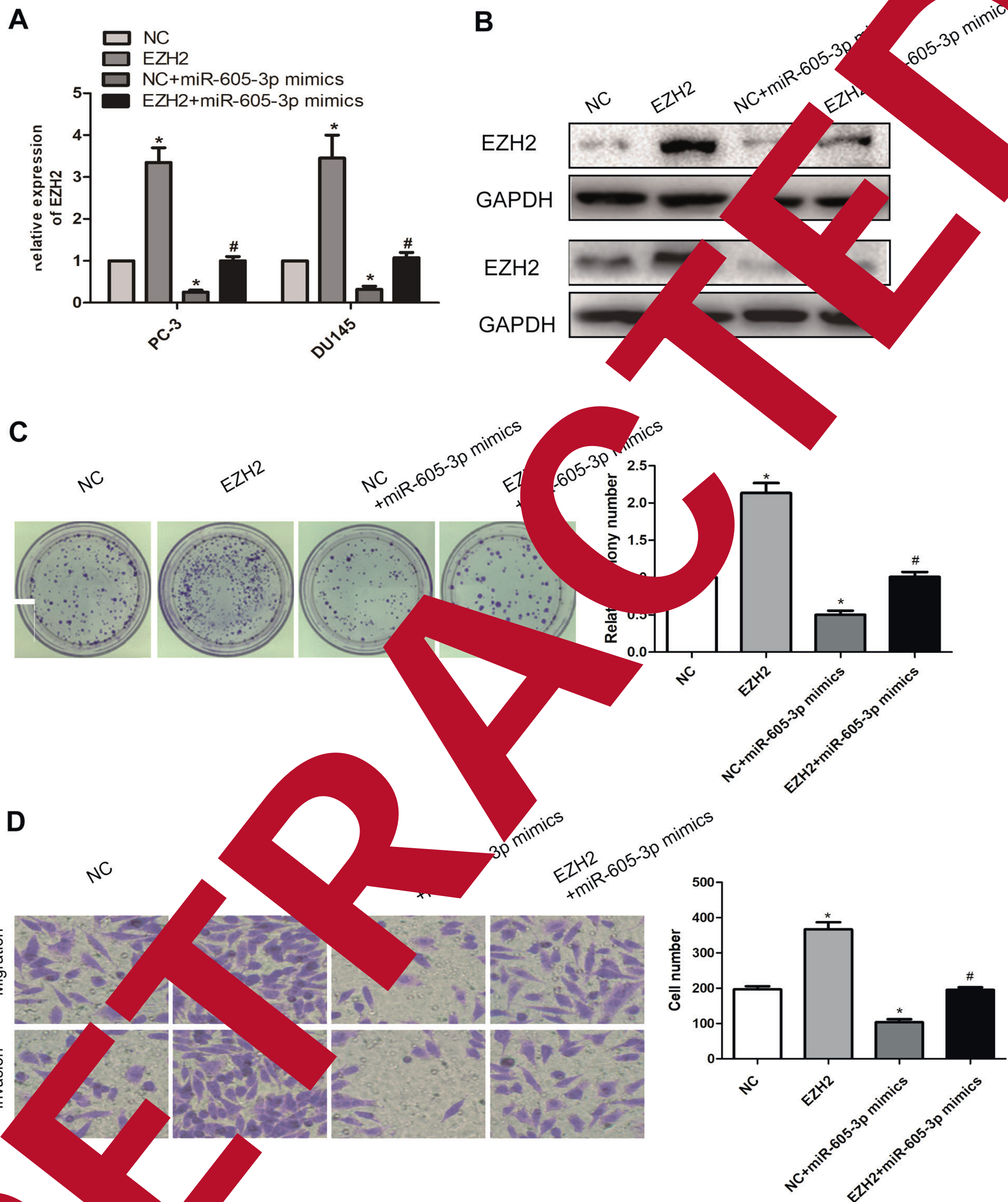


Figure 3. EZH2 is highly express pro ancer tiss cell lines. **A,** qRT-PCR detection of differential expression of EZH2 in prostate cancer tissu d adjace n-tumor ti es. **B,** qRT-PCR detection of EZH2 expression levels in prostate cancer cell lines. **C,** MiR-6 p is signifi ly negatively correlated with the expression level of EZH2 in prostate cancer tissues. **D,** qRT-PCR confirme ressi after transfection of miR-605-3p mimics in PC-3 and DU145 cell lines. Data are shown as m an ± S *p*<0.0 **p<0.001.

nied by chromo py number va ion and rearrangeme . Th stasis and invasion of malignant tumors are ge caused by the destruction invasion of the ent membrane by can cells, which is close related to the dysfu tion of f ctional adhesion of normal epith [28]. De uction and loss of the basement memb cause t basal cell layer and lumen of a tum ells to lose the function ering s, which may cause tumor me sis and d oration[29]. Other studies have sho that the occurrence of prostate cancer is rel onmental and dietary changes. T role of the environment in the oc nce and development of malignant tumors h n gradually recognized by people. With the ovement of living standards of residents, the diet structure of older men has also changed, which has prolonged the life span. It is reported[29] that the incidence of human malignant tumors is closely related to the growth of life expectancy, while the incidence of prostate cancer is gradually increasing with age. Early diagnosis of prostate cancer still relies on molecular immunology and imaging examination, but clinically, prostate cancer has not been effectively treated, especially hormone-resistant prostate cancer (CRPC) is still an important factor threatening the life of prostate cancer patients[30,31]. Therefore, finding new molecular targets for prostate cancer is necessary to guide clinical practice.

MicroRNAs are a class of tiny molecules that function to regulate genes and do not encode proteins. They mainly bind to the 3'-UTR of their



RETRACTED

Fig 4. MiR-605 regulates the expression of EZH2 in prostate cancer tissues and cell lines. **A,** Detection of EZH2 expre on levels in miR-605-3p and EZH2 co-transfected cell lines by qRT-PCR. **B,** Detection of EZH2 in miR-605-3p and EZ d cell lines by Western blot expression level. **C,** Cell colony formation assay is performed to detect the -3p and EZH2 co-transfection on the proliferation of prostate cancer cells. **D,** The transwell migration assay rformed to detect the effect of miR-605-3p and EZH2 co-transfection in regulating the invasion and migration of prostate ells (magnification: 40×). Data are shown as average ± SD, *#p<0.05.

RETRACTED

target genes through complementary pairing principles, leading to the degradation of genes or inhibition of their transcriptional expression[12-14]. Literature has shown that miRNAs are associated with the occurrence and development of various tumors. MiRNAs play a role as oncogenes or tumor suppressor genes[14]. A class of miRNAs is an oncogene in a certain tumor, and is often located in the deletion region or down-regulation of chromosome fragments in the genome, and plays an oncogene-like effect in such cells[13-15]. Deletion of this type of miRNA results in overexpression of its target tumor gene, producing the same effect as tumor gene amplification or activation[15]. The main target gene of another type of miRNA is a tumor suppressor gene, which is often located in the amplified region or expression of the chromosome fragment in the genome, and plays a tumor suppressor-like role[14-16]. Overexpression of this type of miRNA down-regulates the target gene, which is manifested by a decrease in the expression level of the oncogene protein, preventing the malignant transformation of the cell. There are multiple target genes for each miRNA in the cell, while each target gene may interact with m[illegible] miRNAs to form a complex regulatory [illegible] which regulates various genes after trans[illegible]ion and affects tumor development[16]. Although [illegible]R-605-3p has been discovered for a long tim[illegible] biological function has just begun to be studi[illegible] MiR-605-3p acts to inhibit tum[illegible]articipat[illegible] in many physiological and [illegible]ologi[illegible] processes such as bladder cancer[illegible]o better [illegible]derstand miR-605-3p function i[illegible] devel[illegible] progression of PCa, w[illegible] dete[illegible]vel of [illegible]-605-3p in 52 PCa ti[illegible]es and [illegible]ent normal tissues. The expr[illegible]on of 605-3p [illegible]markedly decreased in[illegible] cancer tissu[illegible]nd was strongly asso[illegible]ed w[illegible]a stage, lymph node metastasis and distant m[illegible]sis. Therefore, we believe th[illegible]miR-605-3p may [illegible] role in tumor suppre[illegible]n in PCa. Besides, w[illegible] found that the expr[illegible]on of E[illegible]2 in tumor tissues was significa[illegible]reg[illegible]ed compared with the matched norma[illegible] which w[illegible]n negative correlation with the e[illegible]ion of [illegible]R-605-3p. The above re-[illegible]ere als[illegible]n PCa cell lines. To further inv[illegible]ate the r[illegible] miR-605-3p on the biological f[illegible]ction of PCa, we constructed a miR-605-3p ove[illegible]odel and performed CCK-8, cell [illegible]on experiments, as well as invasion [illegible] migration experiments, which indicated that m[illegible]5-3p can inhibit PCa proliferation as well as in[illegible]sion and migration of PCa; however, its specific molecular mechanism was still unclear. In the current study, we found that [illegible] was markedly down-regulated in pr[illegible]ate canc[illegible] suggesting that miR-605-3p had a[illegible]ential role in prostate cancer.

Next, we sought for the targ[illegible] for miR-605-3p. The eukaryotic g[illegible]ome pr[illegible] thousands of different smal[illegible]NAs (snR[illegible] regulate the expressi[illegible]f various genes [illegible] various conditions, [illegible]ch are [illegible]sponsible for the survival of bio[illegible]bi[illegible] enviro[illegible]ental stresses[12-16]. Res[illegible]ch e[illegible] shows [illegible] RNA and RNA inte[illegible]ence (R[illegible] p[illegible]cipate in the regulati[illegible]f alternative m[illegible]plicing and play a ra[illegible]ion in various [illegible]ell biochemical proc[illegible]es. Its [illegible]nt and versatile regulatory mechanism is the [illegible]omplex cell biology[32]. Evi[illegible]rom bioinfor[illegible] studies[32,33] suggests th[illegible]spe[illegible]fic siRNAs have [illegible]e potential to modu-[illegible]e target gene genes and are likely to play a role [illegible]target gene m[illegible]A cleavage. To clarify the bi-[illegible]ical function [illegible] miRNA, we need to further s[illegible] for its ta[illegible] gene. Bioinformatics analysis [illegible]iR-605-3p inhibited the effect on PCa b[illegible]ng on the 3'-UTR region of EZH2; [illegible]anwhile, if there was a mutation in the 3'-UTR [illegible] inhibition effect would be greatly re-[illegible]ced. [illegible]e expression disorder of EZH2 is close-[illegible]y related to the occurrence and development of a variety of epithelial-derived and hematological tumors, and its high expression can promote the [illegible]nfiltration and metastasis of cancer cells. Therefore, we studied the relationship between EZH2 and prostate cancer[20-22]. It was found that EZH2 can promote the malignant progression of tumor cells in prostate cancer cells.

To study the effect and interaction of miR-605-3p and EZH2 on the development of PCa, we further verified that miR-605-3p mimics significantly down-regulated the mRNA and protein expressions of EZH2. Subsequently, we used a recovery experiment to verify that EZH2 can counteract the ability of miR-605-3p to proliferate, invade and migrate in PCa cells. These above findings suggested that there may be a feedback regulation loop: EZH2 can reverse the biological effects of miR-605-3p in PCa cells, thereby jointly affecting the malignant progression of prostate cancer.

Conclusions

MiR-605-3p was significantly associated with PCa stage, lymph node metastasis and dis-

RETRACTED

tant metastasis and poor prognosis, meanwhile it inhibited the malignant progression of PCa. In addition, miR-605-3p may inhibit PCa proliferation, invasion and migration by regulating EZH2.

Conflicts of interest

The authors declare no conflicts of interest.

References

1) Ritch C, Cookson M. Recent trends in the management of advanced prostate cancer. F1000Res 2018; 7: 10.12688/f1000research.15382.1.
2) Guo T, Wang XX, Fu H, Tang YC, Meng BQ, Chen CH. Early diagnostic role of PSA combined miR-155 detection in prostate cancer. Eur Rev Med Pharmacol Sci 2018; 22: 1615-1621.
3) Ivlev I, Jerabkova S, Mishra M, Cook LA, Eden KB. Prostate cancer screening patient decision aids: a systematic review and meta-analysis. Am J Prev Med 2018; 55: 896-907.
4) Fenton JJ, Weyrich MS, Durbin S, Liu Y, Bang H, Melnikow J. Prostate-specific antigen-based screening for prostate cancer: a systematic eviden[illegible] review for the U.S. Preventive Services Tas[illegible] Rockville (MD), Agency for Healthcare [illegible]arch and Quality (US), 2018; 17-05229-EF-1.
5) Oesterling JE, Martin SK, Bergstralh EJ, Lo[illegible] The use of prostate-specific antigen in sta[illegible] patients with newly diagnosed prostate canc[illegible] JAMA 1993; 269: 57-60.
6) Di Nunno V, Gatto L, Sa[illegible] M, [illegible]amore A, Lopez-Beltran A, Cheng [illegible]carpelli [illegible] Montironi R, Massari F. Recent a[illegible]es in liq[illegible]biopsy in patients with castration[illegible]nt p[illegible] Front Oncol 2018; [illegible]397.
7) Rodriguez JF, Eg[illegible]r SE. Prost[illegible]cer and the evolving role o[illegible]markers in scre[illegible]nd diagnosis. Radio[illegible]th Am 2018; 56[illegible]-196.
8) Sanhueza [illegible]hli M[illegible]al and novel biomarkers in the management of [illegible]te cancer. Curr Treat Optio[illegible]ncol 2018; 19: [illegible]
9) Chi[illegible]ov DA, Myasoedova V[illegible] Grechko AV, Mel[illegible]n[illegible]nko AA, Orekhov AN. New biomarkers for di[illegible]osis and [illegible]ognosis of localized prostate can[illegible] [illegible]mi[illegible]ncer Bio[illegible]2018; 52: 9-16.
10) Wu[illegible] Beretov J[illegible]zi P, Willcox M, Wasinger V, Walsh [illegible]m P[illegible] Urinary biomarkers in pros[illegible]te canc[illegible] and monitoring progression. [illegible] Rev Onc[illegible]matol 2017; 118: 15-26.
11) [illegible]inger P, Hart M, Backes C, Rheinheimer S, Keck [illegible] Keller A, Meese E. Differential blood-[illegible]nosis between benign prostatic hyperplasia and prostate cancer: miRNA as source [illegible]r biomarkers independent of PSA level, Glea[illegible]score, or TNM status. Tumour Biol 2016; 37: [illegible]7-10185.
12) Song CJ, Chen H, Chen LZ, Ru GM, Guo J[illegible] Ding QN. The potential of microRNAs [illegible] prostate cancer biomarkers: a [illegible] ana[illegible] of related studies. J Cell Bioc[illegible] 2018; 11[illegible] 2763-2786.
13) Sole C, Arnaiz E, Lawrie CH. [illegible]RNAs as biomarkers of B-cell lymphoma. [illegible] Insights 2018; 13: 91863240.
14) Pradillo M, Santos JL. [illegible]nes involved [illegible] biogenesis affect m[illegible]sis and fertility. C[illegible]some Res 2018; 2[illegible]33-241.
15) Fuchs WF, Giono[illegible]da [illegible] de la Mata M. Target RNAs strike bac[illegible]RNAs. F[illegible] Genet 2018; 9: 435[illegible]
16) Xie M, M[illegible] Xu T, Pan Y, [illegible]ei Y, Shu Y. Potenti[illegible]atory roles of m[illegible]As and long nonc[illegible]g[illegible] in anticancer therapies. Mol The[illegible]ucleic [illegible]018; 13: 233-243.
17) Zeng Z, Zhou W, [illegible] Zhang J, Lu X, Jin L, Yu [illegible]ular RNA cir[illegible]GL1 as a competing [illegible]genous RNA contr[illegible]utes to bladder cancer progression by regulating miR-605-3p/VANGL1 pathway. J Ce[illegible] Physiol 2019; 234: 3887-3896.
[illegible] Kim KH, Rober[illegible]W. Targeting EZH2 in cancer. Nat Med 2016; 22[illegible]8-134.
19) [illegible]T, Xu [illegible]fects of Enhancer of Zeste Homo[illegible]xpression on brain glioma cell proliferation and tumorigenesis. Med Sci Monit 2018; 24: 7249-7255.
[illegible] Fatkhutdinov N, Fukumoto T, Bitler BG, Park [illegible]ossenkov AV, Trizzino M, Tang HY, Zhang L, Gardini A, Speicher DW, Zhang R. SWI/SNF catalytic subunits' switch drives resistance to EZH2 inhibitors in ARID1A-mutated cells. Nat Commun 2018; 9: 4116.
21) [No authors listed]. MLL1-induced oncogenic reprogramming drives EZH2 inhibitor resistance. Cancer Discov 2018; 8: 1344.
22) Huang X, Yan J, Zhang M, Wang Y, Chen Y, Fu X, Wei R, Zheng XL, Liu Z, Zhang X, Yang H, Hao B, Shen YY, Su Y, Cong X, Huang M, Tan M, Ding J, Geng M. Targeting epigenetic crosstalk as a therapeutic strategy for EZH2-aberrant solid tumors. Cell 2018; 175: 186-199.
23) Yang Y, Liu Y, Li G, Li L, Geng P, Song H. microRNA-214 suppresses the growth of cervical cancer cells by targeting EZH2. Oncol Lett 2018; 16: 5679-5686.
24) Huang B, Huang M, Li Q. MiR-137 suppresses migration and invasion by targeting EZH2-STAT3 signaling in human hepatocellular carcinoma. Pathol Res Pract 2018; 214: 1980-1986.
25) Bjelogrlic S, Todorovic TR, Cvijetic I, Rodic MV, Vujcic M, Markovic S, Araskov J, Janovic B, Emhemmed F, Muller CD, Filipovic NR. A novel binuclear hydrazone-based Cd(II) complex is a strong pro-apoptotic inducer with significant activity against 2D and 3D pancreatic cancer stem cells. J Inorg Biochem 2019; 190: 45-66.
26) Simes BC, Moore JP, Brown TC, Rushforth TJ, Bookout AL, Richardson CL. Genetic polymor-

phism analysis of patients with primary hyperhidrosis. Clin Cosmet Investig Dermatol 2018; 11: 477-483.

27) Wu R, Li H, Peng D, Li R, Zhang Y, Hao B, Huang E, Zheng C, Sun H. Revisiting the potential power of human leukocyte antigen (HLA) genes on relationship testing by massively parallel sequencing-based HLA typing in an extended family. J Hum Genet 2019; 64: 29-38.

28) Li Y, Egranov SD, Yang L, Lin C. Molecular mechanisms of long noncoding RNAs-mediated cancer metastasis. Genes Chromosomes Cancer 2019; 58: 200-207.

29) Ibrahim AM, Sabet S, El-Ghor AA, Kamel N, Anis SE, Morris JS, Stein T. Fibulin-2 is required for basement membrane integrity of mammary epithelium. Sci Rep 2018; 8: 14139.

30) Fujita Y, Kojima K, Ohhashi R, Hamada N, Nozawa Y, Kitamoto A, Sato A, Kondo S, Kojima T, D[illegible] M. MiR-148a attenuates paclitaxel [illegible] hormone-refractory, drug-resista[illegible] prostate cancer PC3 cells by regulating MS[illegible] expression. J Biol Chem 2010; 285: 19076-[illegible]

31) Wang G, Zhao D, Spring DJ, DeP[illegible] Genetics and biology of prostate c[illegible]cer. Ge[illegible] 2018; 32: 1105-1140.

32) Thapar R, Bacolla A, [illegible]niran C, Brickner J[illegible]nam NB, Mosamma[illegible]st N, Tai[illegible] JA. RNA m[illegible]fications: revers[illegible]hanis[illegible] and cancer. Biochemistry 2019; 58[illegible]

33) Hartstock K, [illegible]entmeiste[illegible] Mapping [illegible] -Methyladenosi[illegible] m6A in RNA[illegible] methods, remainin[illegible]llenges and em[illegible] approaches. Chemi[illegible] 25: 3455-3464.

RETRACTED