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miR-564 inhibited metastasis and proliferation of prostate cancer by targeting MLLT3

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Abstract. – OBJECTIVE: MiR-564 has been discovered to be abnormally expressed in human malignancy. Two recent studies suggested that miR-564 plays a role in tumor inhibition in both lung and breast cancer. However, no evidence reported the mechanism and function of miR-564 in prostate cancer (PCa).

PATIENTS AND METHODS: The PCa tissues and their adjacent normal tissues were coll ted from 50 PCa patients. Expressions of m in tissues and cells were evaluated with n CR. The MTT [3-(4,5-dimethylthiazol-2-yl)2 i- phenyl tetrazolium bromide] assay, flow cy etry and Western-blot analysis, were applied detect the proliferation, cell cy rogressio and the protein expression of nes (PC 3 and DU-145). Migration a nvas of PCa cells were analyzed by T well a ys. Furthermore, the correlatio ween MLLT3 was assessed y it por say. Also, the PCa s wer fected with miR-564 mimics c l and inh

RESULTS: In esent resea iR-564 was found dy in PCa cells d to act as a suppres in P ll proliferation, progression c ell cycle, c asion and migration. ML also known as a proto-oncogene, n has first reported n leukemia, and the r ation s expression remains incomple lucid . Also t is first reported in our study g that T3 is a direct target of miR-564 result o showed a significant ive c ti th miR-564 in PCa cells. Fu more, ulation of MLLT3 attenuates the cts of mi 564 on the ability of PCa cells.

CONCLUSIONS: Our research demonstrate- ssor function of miR-564 in PCa, ealing restoration of miR-564 as a potential apeutic strategy for the treatment of PCa.

Key ds:
miR-564, MLLT3/Af9, Prostate cancer.

Intro ion

ostate cancer (PCa) is an important public th problem nd the world. Its incidence ranks 2nd in male tumors, and its fatality ra ks 6th in ale tumors in the world[1]. Beside rate is 28.5/0.1 million people currentl the incidence rate of PCa in China lso shows an increasing trend year by year.

linical symptoms of PCa lack specificit no obvious symptoms in the early stage, but hematuria, lower urinary tract obstruction or irritation symptoms may occur in the progressive stage. The main complications of advanced PCa include the refractory ostealgia, pathological fractures, anemia and spinal cord compression, etc. It is reported that the incidence rate of bone metastases is about 65-75%, and the 5-year survival rate of patients is 25% with poor quality of life[3]. Prostate Specific Antigen (PSA) combined with digital rectal examination is always recognized as the best screening method for early detection of PCa. However, PSA detection still cannot completely distinguish benign from malignant cancer, and PCa cannot be completely ruled out from patients with less than 4 ng/mL antigen, either[4]. PLCO (Prostate, Lung, Colorectal, and Ovarian) study results showed that PSA screening may lead to over-examination and treatment, increased adverse reactions and complications, and higher medical cost, but it cannot reduce the mortality rate of males aged below 55 years old[5]. Therefore, searching for a new screening method of PCa has become one of the research hotspots in recent years. More and more studies have focused on the diagnosis of PCa by detecting the changes in microRNA (miRNA) levels in human blood and

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urine samples. For example, Chen et al[6] identified five miRNAs that were abnormally expressed in the serum of PCa patient for the differential diagnosis of PCa and benign prostatic hyperplasia. Brace et al[7] found that miR-141 and miR-375 are important indexes of prognosis estimation of PCa through the screening of plasma in patients with non-PCa metastasis and PCa metastasis.

MiR-564 has firstly been described as potential blood-based biomarkers in schizophrenia patients together with six other miRNAs[8]. The later reports suggested miR-564 has an inhibitory effect on lung cancer and breast cancer[9,10]. However, the roles of miR-564 in PCa tumorigenesis and underlying mechanisms remain unknown.

MLLT3 (also known as Af9), a proto-oncogene, was first reported in leukemia and involved in many different cellular processes, such as cell differentiation[11], cell fate decision[12] and nervous system development[13]. MLLT3 gene has been reported to play a pathological role in neurodevelopmental diseases. A previous study showed that disruption of MLLT3 was associated with mental retardation, epilepsy and ataxia in human[14]. A later study demonstrated that MLLT3 has p[illegible] tion function in lymphocytoma[15].

In our study, we analyzed the biologica[illegible]n-ction of miR-564 in PCa cells. For the first [illegible] MLLT3 was identified to be a direct and funct[illegible]nal target through which miR-56[illegible]s as tum[illegible] suppressor in PCa cells. Thes[illegible]ndicated that the miR-564 has a tumo[illegible]press[illegible]function in the PCa progress and [illegible]becom[illegible]tial treatment for the PCa.

Patie[illegible]nd Metho

PCa Case[illegible]d Cells

This st[illegible] included 50 [illegible]atients who received [illegible]tment with radica[illegible]rostatectomy at Weif[illegible] Peopl[illegible]Hospital and underwent patho[illegible]l di[illegible]oses to be confirmed as PCa. Preop[illegible]chemot[illegible]py or radiotherapy [illegible]tmen[illegible]e fo[illegible]en. The liquid nitrogen [illegible]ed to[illegible]Ca tissues and correspondin[illegible]jacent n[illegible]l tissues before being kept in -70[illegible]frigerator. The adjacent normal tissues h[illegible]cerned by biological biopsy to be [illegible]e that they do not include cancer cells. This [illegible]y was approved by the Ethics Committee of W[illegible] People's Hospital. Signed written informed [illegible]onsents were obtained from all participants before the study.

The human PCa lines (PC-3 and DU-145) together with the adult human prostatic epithelial cell line (RWPE-1) were purchased from[illegible] nese Academy of Sciences (Shanghai[illegible]). cells were cultured in Roswell Park[illegible]morial Institute 1640 (RPMI 1640) medi[illegible]Thermo Fisher Scientific, Inc., Waltham, M[illegible]A) complemented with 10% fetal [illegible]ine se[illegible]FBS), 100 mg/mL streptomycin[illegible]100 IU/mL[illegible] lin (Thermo Fisher Scie[illegible]c, Inc., Waltham, USA) in 5% CO_2 cel[illegible]ure in[illegible]tor.

qRT-PCR Anal[illegible]

Total RNA[illegible] procure[illegible]TP[illegible] reagent in accordan[illegible]h the manufa[illegible]s protocol. TaqMan [illegible]N[illegible]ay (Applie[illegible] Biosystems, Foster C[illegible], CA, [illegible] was used to measure the le[illegible]of miR-56[illegible]pression normalized to[illegible] U6. SYBR g[illegible] qPCR assay (Life S[illegible]nce, San Diego, CA, USA) was used to me-[illegible]e the level[illegible] MLLT3 expression and en-[illegible]nous contro[illegible] by GAPDH (Life Science, S[illegible]iego, CA[illegible]A).

Weste[illegible]t Analysis

Protein concentration was measured by using [illegible]inic acid (BCA) reagent kit (Merck, [illegible]). Cell lysates were separated on polyacrylamide gels and electro blotted onto nitrocellulose membranes. Then, they were blocked with blocking tris-buffered saline (TBS) with 0.05% Tween 20, pH 7.6 with 5% skimmed milk. After that, the blot was washed and incubated with anti-MLLT3 antibody (Abcam, Cambridge, MA, USA, Dilution 1:1000) or anti-GAPDH (Abcam, Cambridge, MA, USA, Dilution: 1:1000) antibody, which was blotted to show equal protein loading at 4°C overnight.

Transfection

MiR-564 mimics control and inhibitor were synthesized and transfected to PCa cell lines to analyze biological function of miR-564. Then, three groups were established to study the potential relevance between miR-564 and PCa. MiR-564 mimics (PCa cell transfected by miR-564 mimics), mimics +MLLT3 (PCa cell transfected by miR-564 mimics and siMLLT3) and NC group (negative control). All the stuff was purchased from Guangzhou RiboBio (Guangzhou, China), and were transfected by using lipofectamine RNAiMAX (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions.

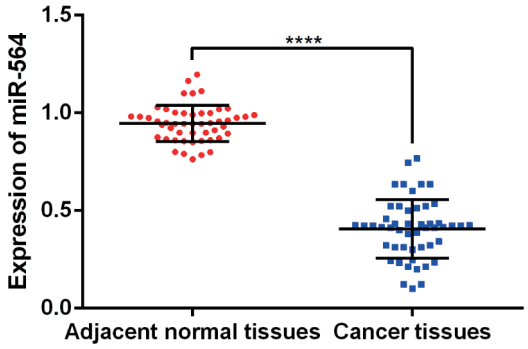

Figure 1. The expressions of miR-564 in prostate tissue samples. Difference in the expression of miR-199a-3p between prostate cancer tissues and corresponding adjacent normal prostate tissues. $^{****}p<0.0001$ compared with RWPE-1.

Cell Proliferation and Cell Cycle Assay

PCa cells were harvested and inoculated into 96-well plates at a density of 2 × 10^3 cells for 48 hours, MTT solution (5 mg/mL, MultiSciences, Hangzhou, China) was appended to each well after 4-hour incubation. Then, 150 µL of dimethyl sulfoxide (DMSO) was added to each well to solubilizing the form azan formed. After 30 minutes, the absorbance was measured by a microplate reader (Bio-Rad, Hercules, CA, USA) set at 490 nm.

For cell cycle analysis, PCa cells were obtained 3 days after transfection. The PCa cells in different cell phase was measured with the cell cycle staining kit (Multi Sciences Biotech Co., Ltd., Hangzhou, China) by flow cytometry. The rate of cells in G0/G1 and S phase presented in the results.

Luciferase Reporter Assays

PCa cells were co-transfected with pMIR-30U-TR-MLLT3 or pMIR-30U-TR-mut MLLT3 and miR-564 mimic or negative control (NC), and the pMIR-Renilla reporter (Promega, Madison, WI, USA) following seeding into a 24-well plate. The cells were then harvested post-transfection. The luciferase activity was assessed using a Dual-Luciferase Reporter Assay System (Promega Corporation, Madison, WI, USA), and results were normalized to Renilla luciferase activity.

Cell Invasion and Migration Assays

Cell invasion and migration assays were performed using Transwell plates (Corning Incorporated, Corning, NY, USA) with 8-µm-pore size membranes with Matrigel (for invasion assay) or without Matrigel (for migration assay). Briefly, 2 × 10^4 cells were planted into the upper chambers with serum free medium, On the other hand, the lower chamber was offered with medium containing 10% fetal bovine serum (FBS) as a chemoattractant. After 2 days incubating, the cells on the top of membrane were wiped off with a brush. Subsequently, the membrane was stained with 0.2% crystal violet followed dried by 95% ethanol. The cells of migrating and invading were noted under an inverted microscope.

Statistical Analysis

Statistical analysis was performed with a Student's *t*-test or Mann-Whitney test. All *p*-values were two-sided and *p* < 0.05 was considered statistically significant and analyzed using Prism software (Version X; La Jolla, CA, USA).

Results

miR-564 Expression Found Reduced in Tissues and Cells of PCa

To explore the role of miR-564 in PCa, we used qRT-PCR to detect the expression level of miR-564 in PCa tissues and the adjacent normal tissues. The results showed that the expression of miR-564 is pretty lower in PCa tissues compared with the adjacent normal tissues ($p<0.05$) (Figure 1). Furthermore, we found the same results in PCa cells compared to the RWPE-1 cell ($p<0.05$) (Figure 2). Together, we thought miR-564 might have the regulatory effects in PCa progression.

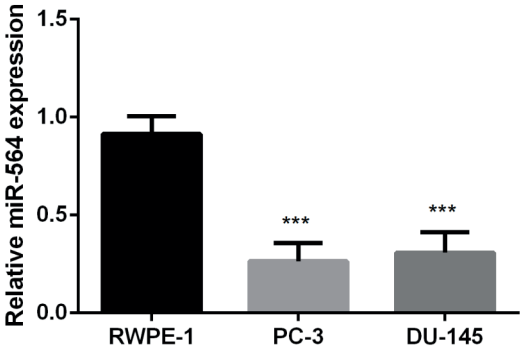

Figure 2. The expressions of miR-564 in prostate cells. The expression of miR-199a-3p in prostate cancer cell lines (PC-3 and DU145) and normal prostate epithelial cells (RWPE-1). $^{***}p<0.001$ compared with RWPE-1.

MiR-564 Suppressed Proliferation of PCa cells

To examine the function of miR-564 on proliferation of PCa cells, we performed MTT assay to detect the cell proliferation rates. The MTT results showed that up-regulation of miR-564 significantly decreased cell proliferation rates in both PC-3 and DU-145 cells. In contrast, down-regulation of miR-564 promoted cell growth of PCa cells ($p<0.05$) (Figure 3A, B). In order to understand the underlying mechanism, we make a further experiment to explore whether the miR-564 influences on cell cycle progression. We found that overexpression of miR-564 significantly increased the percentage of cells at G0/G1 phase. However, inhibition of miR-564 exhibited an opposite effect ($p<0.05$) (Figure 3C, D). As a result, the present study suggested that miR-564 inhibited PCa cell growth via inducing cell-cycle arrest at G0/G1 phase.

MiR-564 Inhibited Migration and Invasion of PCa Cells

Migration and invasion are two most key factors in cancer cell proliferation. In the Tra... experiments, results showed that migrat... invasion in both PC-3 and DU-145 cells w... stricted after up-regulation of miR-564 wit... mics. However, inhibition of miR-564 resulte... promotion of migration and inva... in PCa cel... ($p<0.05$) (Figure 3G-H).

MLLT3 is a Direct Tar... of miR... in PCa Cells

In order to explore ...ntia... of miR-564, we made a predicti... ...ia searchi... ...e publicly available algori... Target Scan, ...DB and microRNA) t... ...cid... ...e putative an... possible targets of mi...64. Fina... found the MLLT3 was a su... ...ed target of ...564 (Figure 4A) (564c) ...cent study has de... ...nstrated that the MLL... has th... ...nction of promotion in lymph... ...ma[15] ...s, MLL...3 has caught our attention. ... whet... ...iR-564 has regulation ...ct on ...T3, ...y, we established lucife... ...porter ... containing the wild or mutant ...e miR-... ...eed sequences of the MLLT3 30U... Increase the expression of miR-564 with ... ...n the decrease of the luciferase ...vity of the wide type MLLT3 30UTR reporter ... but it has no effect on mutant-type (Figure 4... ...thermore, we found that up-regulation of miR-...64 decreases the expression level of protein of MLLT3 in both PC-3 and DU-145 cells in Western blot experiment (Figure 3E-F). Secondly, we explored the correlation between MLLT3 and miR-564 on PCa cells. We set up three ... conduct the similar experiments (mi... ...g... miR-564 mimics group, and the mi... ...+ MLLT3 group) in PC-3 cells. As we exp... ...restoration of MLLT3 has the reverse force on ...pressing effect induced by miR-564 ... the p... ...ation of PCa cells (Figure 4B) ... cycle pro... (Figure 4D), protein ex... ...sion (F... ...ure 4E) ... gration, and invasion ... ...ure 4F... ...ken it all, the results indicated that ... ...p... ...lation ... miR-564 has a negat... ...rela... ... with ...LT3 in PCa cell, miR... suppress... ...n... ...tasis and proliferation ...a cells, and ... ...restoration partially ...v... ...el... ...hibition of ...R-564.

Discus...n

...Ca is a maj... ...blic health problem in deve... ...d countries[1]. ...raditional treatments of PCa r... ...hormon... ...rapy, but the cancer cells gradua... ...sensitive and resistant to hormone tre... ...t as the disease progress[17]. Thus, it ... important to explore the pathologic process of ...development. Loss of cycle control is an ...po... ...t pathological factor in the occurrence of tumors including PCa. Cell cycle disorder is an important cause for unstable genetic information and malignant cell transformation[18]. Studies have showed a large number of cycle regulatory proteins that are abnormally expressed in PCa. Many cycle regulation- associated proteins will mutate in PCa, which may lead to the activation of oncogenes and inactivation of tumor-suppressor genes[19]. The pharmacological basis of a lot of therapeutic drugs of PCa, including the existing chemotherapy drugs, is the cell cycle regulation[20]. Therefore, the study on the new cycle regulatory genes will undoubtedly bring breakthroughs to the development of new targeted drugs of PCa. At present, studies have demonstrated that miRNAs play an important role in the transformation of stem cells from G1 phase to S phase[21], but the G1 phase of tumor cells is short and G1/S detection sites are lacked. At this point, tumor cells and embryonic stem cells are similar. There are many miRNAs that inhibit the tumor proliferation by regulating cell cycle. For example, miR-765 arrests the cell cycle in G2 phase via inhibiting HMGA 1[22]. MiR-107 and miR-449a can induce the G1 phase arrest by targeting CDK4/CDK6[23]. This phenomenon suggests that miRNA may be closely related to

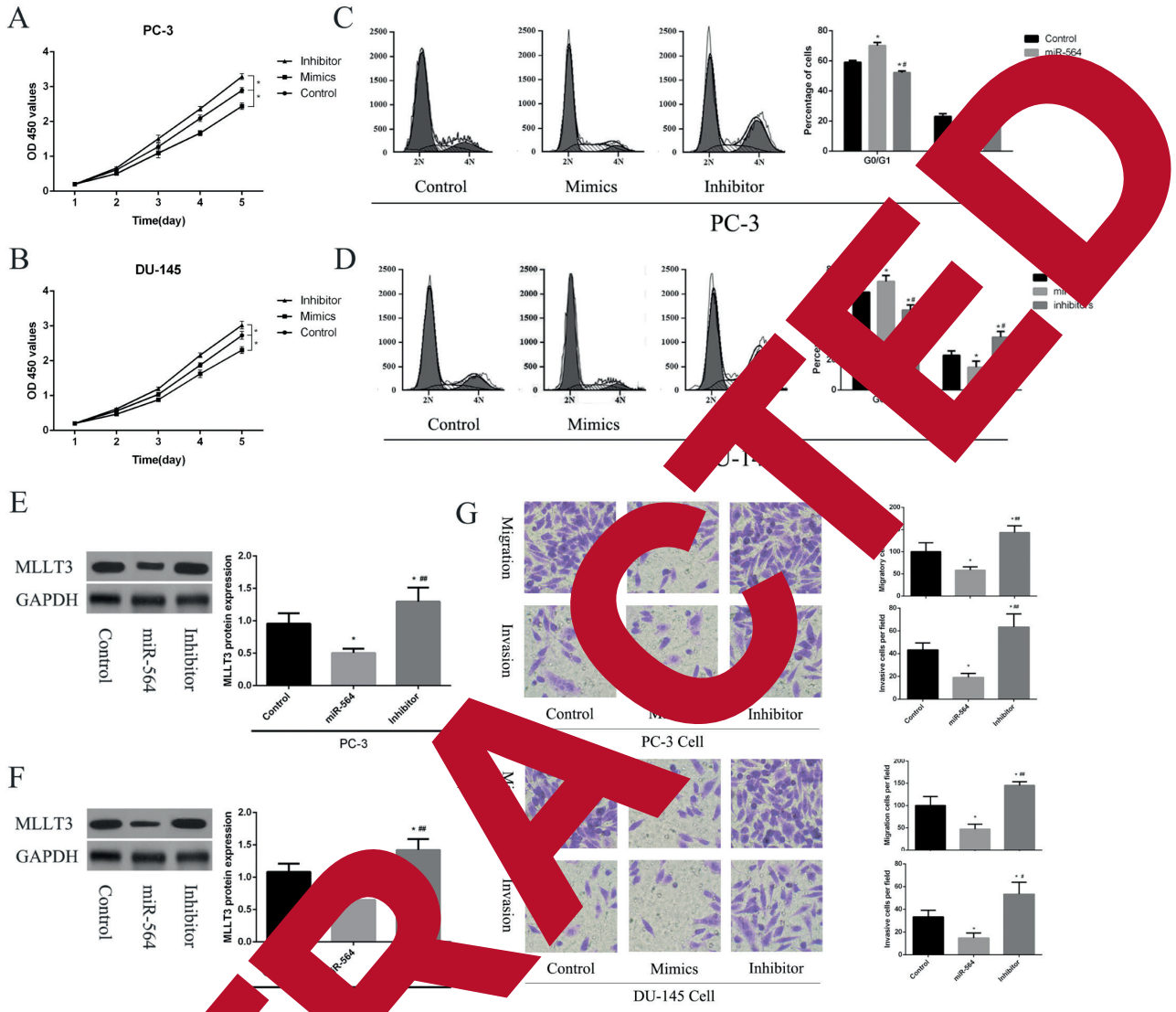

Figure 3. MiR-564 i… the prolifera… motility of prostate cancer cells. PC-3 and DU-145 cells were transfection with miR-564 mimics a… r. ***A-B,*** Effect … 564 on the cell proliferation. The cell proliferation of PCa cell transfection with mimics or in… alyzed using M… assay. (*p<0.05 *vs.* control group). ***C-D,*** Effect of miR-564 on the cell cycle. The cell cycle p… s of PCa… nsfection with mimics or inhibitor were analyzed using flow cytometry, (*p<0.05 *vs.* control group; # p<0… *s.* mimics gro… ***E,*** Effect of miR-564 on the expression of MLLT3. The protein expression of MLLT3 of PCa cell p… ansfection with mi… nhibitor were analyzed using western-blot assay (*p<0.05 *vs.* control group; ##p<0.01 *vs.* mim… oup). ***G-H,*** Effect of mi… 64 on the invasion and metastasis. The invasion and metastasis of PCa cell post-transfecti… h mimi… inhibitor were analyzed using transwell assay and detected by microscope (×200). (*p<0.05 *vs.* control gro… <0.05, … 0.01 *vs.* mimics group).

…nor ce… …gulation. All these cycle-related … mor sup… sor microRNAs are expected to l… therapeutic targets for PCa. Here in our s… …zed samples from 50 PCa patients. These results indicated that compared with …sponding adjacent normal tissues, miR-564 e… …ion was significantly down-regulated in PCa. We also found that expressions of miR-564 in PC-3 and DU-145cell lines were significantly decreased compared with RWPE-1 cells, suggesting that miR-564 has a significant effect on PCa development. Besides, over-expressed miR-564 inhibited the proliferation of PCa cells and had the function of inducing cell-cycle dispute at G0/G1 phase. The invasion and metastasis of prostate cancer is a multi-step and multi-factor complex process, involving a variety of adhesion-related molecules, proteolytic enzymes, many cytokines

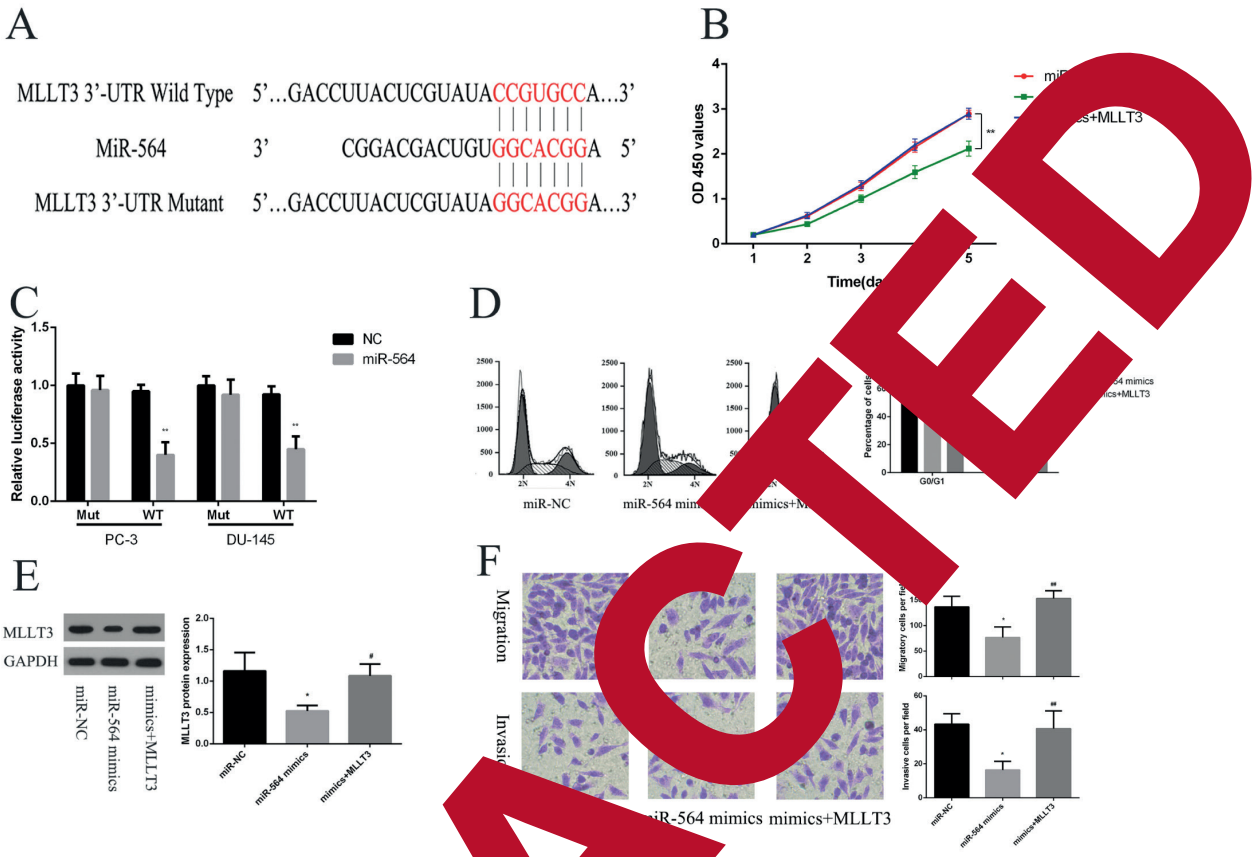

Figure 4. MLLT3 is a direct and functional target of [illegible] MLLT3 overexpression attenuates the suppressive effect of miR-564 on PC-3 cell. ***A,*** Diagram of putative miR-564 [illegible] sites of MLLT3. ***B,*** Cell proliferation test by MTT assay. ***C,*** Relative activities of luciferase report[illegible] cycle pro[illegible]on test by flow cytometry. ***E,*** Protein levels of MLLT3 test by western-blot assay. ***F,*** The invasion [illegible] test by tr[illegible]ll assay. ($^{*}p<0.05$, $^{**}p<0.01$ *vs.* NC group; $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs.* mimics group).

and regulatory fac[illegible]. More a[illegible]re eviden-ce suggests tha[illegible]A is also c[illegible]related to the metast[illegible]of [illegible]. For example, the sup-pressor gen[illegible]of tumor, p[illegible]an inhibit the tumor metastasi[illegible] regulating t[illegible]ression of miR-205, a[illegible]tients with p-63/mi[illegible]205 deletion are often [illegible]compar[illegible]with high Gleason score and ea[illegible]nor m[illegible]stasis[25]. Our findings suggested that t[illegible]of mig[illegible]y and invasive of PCa [illegible]s wer[illegible]icted [illegible]p-regulation of miR-564, [illegible]ting t[illegible]564 has a certain inhibitory effe[illegible]n tumor [illegible]liferation.

S[illegible]uently, we searched the potential targets t[illegible] miR-564 exerted its effect on PCa [illegible]s. MLLT3 aroused our interest after it was [illegible]yzed in three online bioinformatics databases, w[illegible]have been suggested to have the function of p[illegible]motion in malignancy[15]. As we expected, we found that the expression of miR-564 nega-tively correlated with the expression of MLLT3 utilizing in luciferase assay and Western blot. Mo-reover, MLLT3 overexpression seriously impacts the inhibitive function of miR-564 on PCa cells in cell proliferation, cell cycle, the invasion and me-tastasis of cell, indicating that miR-564/ MLLT3 axis may be an important mechanism in the tumo-rigenesis and development of PCa.

Conclusions

We explored the tumor inhibitory function of miR-564 in PCa cells. MLLT3, as the direct and function target of miR-564, attenuated the sup-pressor effect in metastasis and proliferation of PCa cell. Thus, restoration of miR-564 could be a potential therapeutic strategy for the treatment of PCa.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Xu CG, Yang MF, Fan JX, Wang W. MiR-30a and miR-205 are downregulated in hypoxia and modulate radiosensitivity of prostate cancer cells by inhibiting autophagy via TP53INP1. Eur Rev Med Pharmacol Sci 2016; 20: 1501-1508.
2) Di Franco R, Borzillo V, Ravo V, Ametrano G, Cammarota F, Rossetti S, Romano FJ, D'Aniello C, Cavaliere C, Iovane G, Porricelli MA, Muto M, Berretta M, Facchini G, Muto P. rectal/urinary toxicity after hypofractionated vs. conventional radiotherapy in high risk prostate cancer: systematic review and meta analysis. Eur Rev Med Pharmacol Sci 2017; 21: 3563-3575.
3) Smith JJ, Soloway MS, Young MJ. Complications of advanced prostate cancer. Urology 1999; 54: 8-14.
4) Crawford ED, Moul JW, Rove KO, Pettaway CA, Lamerato LE, Hughes A. Prostate-specific antigen 1.5-4.0 ng/mL: a diagnostic challenge and danger zone. BJU Int 2011; 108: 1743-1749.
5) Andriole GL, Crawford ED, Grubb RR, Buys SS, Chia D, Church TR, Fouad MN, Gelmann EP, Kvale PA, Reding DJ, Weissfeld JL, Yokochi LA, O'Brien B, Clapp JD, Rathmell JM, Riley TL, Hayes RB, Kramer B[illegible]lian G, Miller AB, Pinsky PF, Prorok PC, Go[illegible] Berg CD. Mortality results from a randomiz[illegible]state-cancer screening trial. N Engl J Med [illegible] 360: 1310-1319.
6) Chen ZH, Zhang GL, Li HR, Luo J[illegible] ZX, Chen G[illegible] Yang J. A panel of five circu[illegible]RNAs as potential biomarkers for pr[illegible]e ca[illegible] Prostate 2012; 72: 1443-1452.
7) Brase JC, Johannes M, S[illegible] T, Fa[illegible] Steuber T, Beissbarth [illegible] Ku[illegible]nn H. C[illegible]lating miRNAs ar[illegible]elate[illegible]mor progression in prostat[illegible]cer. Int J C[illegible] 2011; 128: 608-616.
8) Lai CY, Yu [illegible] Chen CH, Ch[illegible] HY, Wen CC, Huang [illegible], Hsiao [illegible] CK, Liu CM, Yang PC, Hwu H[illegible]hen WJ. Mic[illegible] expression aberration [illegible]otential periphera[illegible] biomarkers for sc[illegible]hrenia. PLoS One 20[illegible], 6: e21635.
9) [illegible] B, Jia [illegible] Q, Ren H, Hu D, Zhou X, Ren Q, [illegible] Xie [illegible]-564 f[illegible]tions as a tumor suppres-s[illegible] lung [illegible]r by targeting ZIC3. Biochem[illegible]ys Re[illegible]mmun 2015; 467: 690-696.
10) [illegible]tlu M[illegible]nsari SA, Yurdusev E, Shehwana [illegible] Konu O, [illegible]U, Sahin O. MiR-564 acts as a [illegible]l inhibitor o[illegible] PI3K and MAPK signaling networ[illegible]its proliferation and invasion in breast ca[illegible] Rep 2016; 6: 32541.
11) Pina C, May G, Soneji S, Hong D, Enver T. MLLT3 re[illegible]ulates early human erythroid and megakaryocytic [illegible] fate. Cell Stem Cell 2008; 2: 264-273.
12) [illegible]ogel T, Gruss P. Expression of Leukaemia associated transcription factor Af9/Mllt3 in the cerebral cortex of the mouse. Gene Expr Patterns 2009; 9: 83-93.
13) Buttner N, Johnsen SA, Kugler S, Vogel T. [illegible]Mllt3 interferes with Tbr1 expression throu[illegible] tic modification of histone H3K79 d[illegible]g develo[illegible]ment of the cerebral cortex. Proc [illegible] Acad Sci U S A 2010; 107: 7042-7047.
14) Pramparo T, Grosso S, Messa J, Zattera[illegible]aglia MC, Chessa L, Balestri P, Rocchi [illegible] Zuffardi [illegible]da R. Loss-of-function mutation[illegible]e AF9/MLL[illegible] a girl with neuromotor d[illegible]opment delay, ce[illegible] ataxia, and epilepsy. [illegible] Genet 2[illegible] 118: 76-8[illegible]
15) Zhang T, Luo Y[illegible]g T[illegible]ng JY. MicroRNA-297b-5p/3p targ[illegible] to sup[illegible]s lymphoma cell p[illegible]ration, [illegible]tion an[illegible]asion in vitro and t[illegible] growth in [illegible]mi[illegible]euk Lymphoma 2[illegible]: 2033-2040.
16) Zhang [illegible]D, Xie HF, We[illegible]X. Breast cancer m[illegible]stasis s[illegible]sor 1 (BRMS1) suppresses prostate cancer pr[illegible]on by inducing apoptosis [illegible]ulating invasi[illegible] Rev Med Pharmacol [illegible] 2017; 21: 68-75.
17) Sturge J, Caley MP, Waxman J. Bone metastasis in prostate can[illegible] emerging therapeutic strategies. Nat Rev Clin [illegible]l 2011; 8: 357-368.
18) [illegible]erty SC, [illegible]own SR, McKelvey-Martin V, Dow[illegible] Yoo JJ, Simpson DA, Kaufmann WK. Ce[illegible]eckpoint function in bladder cancer. J Natl Cancer Inst 2003; 95: 1859-1868.
19) [illegible] JT, Lehmann BD, Terrian DM, Chappell WH, Stivala [illegible]a M, Martelli AM, Steelman LS, McCubrey JA. Ta[illegible]geting prostate cancer based on signal transduction and cell cycle pathways. Cell Cycle 2008; 7: 1745-1762.
20) Diaz-Moralli S, Tarrado-Castellarnau M, Miranda A, Cascante M. Targeting cell cycle regulation in cancer therapy. Pharmacol Ther 2013; 138: 255-271.
21) Ward EJ, Shcherbata HR, Reynolds SH, Fischer KA, Hatfield SD, Ruohola-Baker H. Stem cells signal to the niche through the Notch pathway in the Drosophila ovary. Curr Biol 2006; 16: 2352-2358.
22) Leung YK, Chan QK, Ng CF, Ma FM, Tse HM, To KF, Maranchie J, Ho SM, Lau KM. Hsa-miRNA-765 as a key mediator for inhibiting growth, migration and invasion in fulvestrant-treated prostate cancer. PLoS One 2014; 9: e98037.
23) Liang LH, He XH. Macro-management of microRNAs in cell cycle progression of tumor cells and its implications in anti-cancer therapy. Acta Pharmacol Sin 2011; 32: 1311-1320.
24) Hudson BD, Kulp KS, Loots GG. Prostate cancer invasion and metastasis: insights from mining genomic data. Brief Funct Genomics 2013; 12: 397-410.
25) Tucci P, Agostini M, Grespi F, Markert EK, Terrinoni A, Vousden KH, Muller PA, Dotsch V, Kehrloesser S, Sayan BS, Giaccone G, Lowe SW, Takahashi N, Vandenabeele P, Knight RA, Levine AJ, Melino G. Loss of p63 and its microRNA-205 target results in enhanced cell migration and metastasis in prostate cancer. Proc Natl Acad Sci U S A 2012; 109: 15312-15317.