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RLIP76-dependent suppression of PI3K/AKT/Bcl-2 pathway by miR-101 induces apoptosis in prostate cancer

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ABSTRACT

MicroRNA-101 (miR-101) participates in carcinogenesis and tumor progression in various cancers. However, its biological functions in prostate cancer are still unclear. Here, we demonstrate that miR-101 represents a critical role in regulating cell apoptosis in prostate cancer cells. We first demonstrated that miR-101 treatment promoted apoptosis in DU145 and PC3 cells by using flow cytometric analysis and transmission electron microscopy (TEM). To verify the mechanisms, we identified a novel miR-101 target, Ral binding protein 1 (RLIP76). We found miR-101 transfection significantly suppresses RLIP76 expression, which can transactivate phosphorylation of PI3K-Akt signaling, and resulted in an amplification of Bcl2-induced apoptosis. Furthermore, we demonstrated that RLIP76 overexpression could reverse the anti-tumor effects of miR-101 in DU145 and PC3 cells by using flow cytometry assay and MTT assay. Taken together, our results revealed that the effect of miR-101 on prostate cancer cell apoptosis was due to RLIP76 regulation of the PI3K/Akt/Bcl-2 signaling pathway.

1. Introduction

Prostate cancer represents one of the most common cancers in men [1]. With the development of early diagnosis and treatment modalities, the 5-year survival rate of prostate cancer has been improved over the past two decades [2]. However, even with aggressive intervention (including surgery, radiotherapy and chemotherapy), it does not ensure a cure especially for those castration-resistant prostate cancers (CRPC) [3]. To improve the prognosis of patients with malignant prostate cancer, it is urgent to identify new molecular biomarkers that regulate malignant biological behavior of prostate cancer cells.

MicroRNAs (miRNA), a class of nocoding RNA molecules, negatively mediate the translation of mRNAs by interacting with complementary sites in the 3′ untranslated region (3′ UTR) [4]. The close relationship between miRNAs and cellular functions, such as cell growth, migration, and cell cycle, are well documented [5]. They also participate in several pathological processes of cancers [6]. Some miRNAs have been regard as suppressor genes in specific cancers by directly targeting oncogenes [7]. Mounting evidences indicate that microRNA-101 (miR-101) is a potential cancer marker and functions as a tumor suppressor gene in several tumors. Specially, aberrant low expression level of miR-101 has been demonstrated in a variety of malignancies, such as lung, colon and bladder cancers [8–10]. Furthermore, recent studies have demonstrated that miR-101 decreases the invasiveness and regulates cell proliferation in prostate cancers, highlighting its importance in prostate cancer [11,12]. However, the molecular mechanisms of miR-101 in cell apoptosis in prostate cancer cells are still unknown.

Ral binding protein 1 (RLIP76), a GTPase-activating protein, is required for tumor progressions, including cell proliferation, apoptosis and invasion [13–15]. Previous studies have shown that the suppression of PI3K/Akt signaling has been observed together with the downregulation of RLIP76 in pancreatic cancer. Consistent with its growth-promoting role, RLIP76 is regarded as an oncogene [16]. Importantly, excessive expression of RLIP76 is positively correlated with tumor growth in prostate cancer xenografts, suggesting that increased RLIP76 level may be critical to prostate cancer patient survival. Here, we confirmed the function of miR-101 in prostate cancer cell apoptosis and demonstrated that miR-101 suppresses PI3K/Akt pathway via directly targeting the multifunctional transporter protein RLIP76.

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2. Material and methods

2.1. Transfection and luciferase assay

The miRNA transfection and Luciferase assay were performed as described previously [17]. Briefly, human prostate cell line DU145 and PC3 were transfected 24 h later using Lipofectamine 2000 according to the manufacturer's instructions (Life technology, USA). MiR-101 precursor molecules (Ambion, USA) were co-transfected with 4 ng/well of plasmid pRL-CMV and 80 ng/well of luciferase reporter plasmids.

2.2. Tissue samples

The study protocol complies with National Regulations on the Use of Clinical Samples in China. Specialty Committee on Ethics of PLA General Hospital approved human specimen use in this study. Prostate cancer specimens were obtained from patients with surgery at the PLA General Hospital from July 2008 to July 2012.

2.3. Constructs and transfection

The synthetic interfering RNA (siRNA) sequences and the full length of RLIP76 were carried out using pLVTHM vector as described previously and purity was confirmed by Western blot analyses [13]. The DU145 and PC3 cells were stably transfected with GFP-siRNA, or RLIP76-siRNA according to the manufacturer's protocol (Invitrogen). Silencing of target gene expression was evaluated 3 d following transfection and did not decrease significantly by 9 d after transfection as determined by Western blot analysis.

2.4. Western blotting

Western blot analyses were conducted as described previously [13]. The primary antibodies were RLIP76 (Abcam, ab56815, 1:1000), PI3K (Sigma Aldrich, SRP5319, 1:800), Bcl-2 (Sigma Aldrich, SAB4300339, 1:500), AKT (Santa Cruz, sc-8312, 1:800), or β-actin (Santa Cruz, sc-8432, 1:500).

2.5. Quantitative RT-PCR analysis

RNeasy mini kit (Qiagen) was used to extract total RNA from prostate cancer cell lines and frozen tissue specimens. The primers for RLIP76, Bcl-2, PI3K, AKT and β-actin were designed as follows:

RLIP76 5′-ACGGAGACTGAGAAAGTGCAGGAA-3′ forward, 5′-GTCCATGTGCACAATGAGCCAAGA-3′ reverse; Bcl-2 5′-CCGGGAGATCGTGATGAAGT-3′ forward, 5′-ATCCCAGCCTCCGTTATCCT-3′ reverse; PI3K, 5′-CATCACTTCCTCCTGCTCTAT-3′ forward, 5′-CAGTTGTTGGCAATCTTCTTC-3′ reverse; AKT, 5′-GGACAACCGCCATCCAGACT-3′ forward, 5′-GCCAGGGACACCTCCATCTC-3′ reverse; β-actin, 5′-ACTCGTCATACTCCTGCT-3′ forward, 5′-GAAACTACCTTCAACTCC-3′ reverse ;

2.6. MTT assay

MTT assay was performed as previously described [13]. Cell viability was demonstrated by MTT assay at daily intervals. After removal of the medium, dimethyl sulfoxide (DMSO) dissolved the formazan crystals. The absorbance was examined at 570 nm.

2.7. Apoptosis assay

Annexin V-FITC apoptosis kit was used to measure apoptosis according to the manufacturer's instructions (Immunochemistry technologies). Cells were analyzed by flow cytometry after stained with anti-Annexin V-FITC antibody.

2.8. Transmission electron microscopy (TEM)

The fixed material was dehydrated in a graded alcohol series and embedded in Epon 812. Images were captured from a 12 bits CCD camera. For TEM, ultra-thin sections were classically contrasted with Ultracut S and then stained with uranyl acetate and lead citrate for 30 and 5 min, respectively [13]. The stained sections were observed with a Philips 400 transmission electron microscope at 60 kV.

2.9. Statistics

The $\chi 2$ test was used to associate the qRT-PCR data and clinicopathological parameters. Cox proportional-hazards regression analysis was used to evaluate predictors of survival. Kaplan–Meier survival analysis was used to analyze overall survivals in prostate cancer patients. A two-tailed P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Apoptosis is induced by miR-101 in prostate cancer cells in vitro

We first established DU145 and PC3 cell line transfected with miR-101, non-effective control (miR-NC) or mock (control) to explore the effects of miR-101 on prostate cancer cell apoptosis. Transfection of DU145 and PC3 cells with miR-101 led to a significant amplification in miR-101 expression in DU145 and PC3 cells compared to miR-NC and mock cells (Fig. 1A). Next, we found miR-101 significantly suppressed cell proliferation in both DU145 and PC3 cells by using MTT assay (Fig. 1B), which was consistent with previous results [12]. In addition, flow cytometric analysis indicated that the apoptosis was significantly enhanced in DU145 and PC3 cells expressing miR-101 compared to cells transfected with miR-NC or mock (Fig. 1C). Moreover, we used TEM analysis to confirm in evaluation that annexin V stain showed the apoptotic changes in DU145 and PC3 cells transfected with miR-101. The miR-101-transfected cells revealed significant typical apoptosis characteristics, such as nuclear fragmentation, membrane blebbing and chromatin condensation compared with control cells (Fig. 1D), strongly suggesting that miR-101 plays a critical role in regulating prostate cancer cell apoptosis in vitro.

3.2. RLIP76 is a novel target of miR-101

We speculate that Ral binding protein 1 (RLIP76) is a potential target of miR-101 because it contains a putative miR-101 target site in its 3′UTR by using target scan. We cloned the target site, or its mutant into an identical luciferase reporter vector (Fig. 2A). We found that the reporter vectors with the putative target sequence resulted in an approximately 38.34 ± 3.1% decrease in relative luciferase activity compared to the mutant introduced with miR-101 in DU145 cells (Fig. 2B). Furthermore, miR-101 transfection significantly decreased both the mRNA and protein level of RLIP76 in DU145 and PC3 cells (Fig.2C). In addition, we measured the expression of RLIP76 and miR-101 in 32 prostate cancer specimens to investigate its clinical relevance in vivo. We found the mRNA expression of RLIP76 was significantly increased in cancers compared to paraneoplastic or normal group by using qRT-PCR (Fig. 2D). Furthermore, statistical analyses of mRNA expression demonstrated that RLIP76 expression associated inversely with miR-101 expression ($r = -0.365$, $p = 0.0398$) (Fig. 2E). Thus, these results indicated that RLIP76 is a novel target of miR-101.

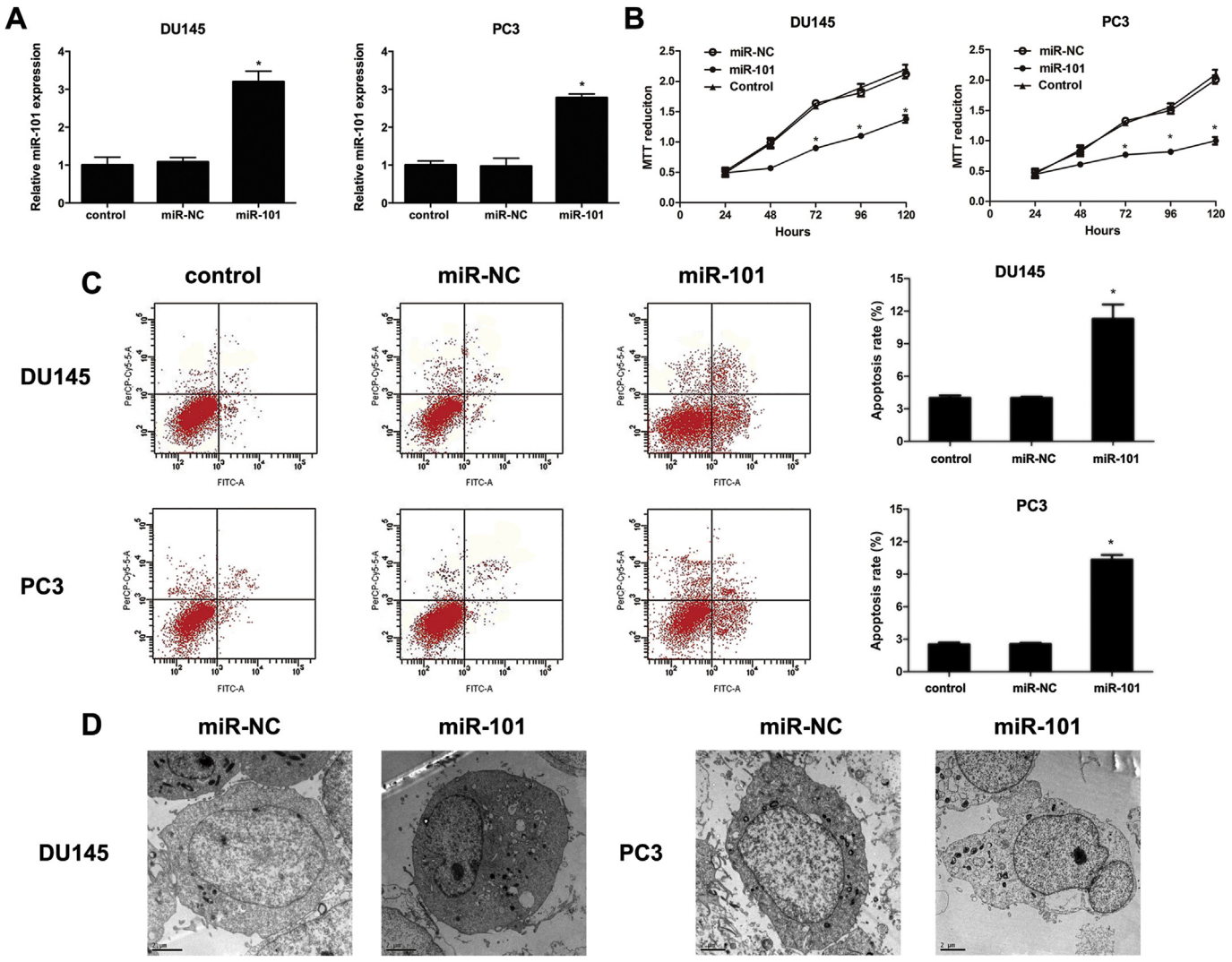

Fig. 1. (A) miR-101 expression was significantly increased in DU145 and PC3 cells transfected with miR-101 compared to cells transfected with miR-NC. $^{*}P < 0.01$ (B) miR-101 significantly suppressed cell proliferation in cells expressing miR-101 compared to controls. $^{*}P < 0.05$ (C) miR-101 significantly enhanced cell apoptosis in cells expressing miR-101 compared to controls. $^{*}P < 0.05$ (D) The miR-101-transfected cells revealed typical apoptosis features, such as nuclear fragmentation, chromatin condensation and membrane blebbing.

3.3. Knockdown of RLIP76 enhances apoptosis in human prostate cancers

Given that administration of RLIP76 siRNA to mice bearing xenografts of PC-3 prostate cancer cells leads to near complete regression of xenografts, we hypothesized that RLIP76 might promote tumor progression by suppressing apoptosis [18]. To ascertain this, we first measured RLIP76 expression in four meningioma cell lines (PC3, DU145, LNCaP, and PrEC) by using Western blot (Fig. 3A). PC3 and DU145 cells showed significantly higher RLIP76 expression than LNCaP and PrEC cells. So PC3 and DU145 cells were chosen to perform the next experiments in vitro. PC3 and DU145 cells transfected with RLIP76 siRNA exhibited a significant reduction in RLIP76 expression by Western blot (Fig. 3B). We then measured the contribution of RLIP76 expression to the PC3 and DU145 cell apoptosis by flow cytometric analysis. Knockdown of RLIP76 significantly enhanced cell apoptosis in PC3 and DU145 cells transfected with RLIP76 siRNA compared to cells transfected with control siRNA (Fig. 3C). These results indicated that RLIP76 could suppress apoptosis in human prostate cancer cells.

3.4. Apoptosis induction by miR-101 is RLIP76–dependent

To test whether RLIP76 is a mediator in miR-101-induced apoptosis, full-length RLIP76 was transfected into DU145 and PC3 cells treated with miR-101. We confirmed that miR-101 significantly decreased RLIP76 protein levels and this effect could be in part alleviated by RLIP76 transfection in prostate cancer cells by Western blot analysis (Fig. 3D). MTT assay and apoptosis assay showed that overexpression of RLIP76 in DU145 cells expressing miR-101 reversed the enhanced apoptosis and lower growth observed in cells expressing only the miR-101 (Fig. 3E, F). Similar results were observed in PC3 cells (Fig. 3E, F). These results imply that miR-101 induces apoptosis through an RLIP76–dependent mechanism.

3.5. Suppression of PI3K/Akt activation by miR-101 is regulated by RLIP76

To investigate possible downstream signaling related to miR-101-induced apoptosis, PI3K and Akt status were examined by Western blot assay in DU145 and PC3 cells transfected with miR-

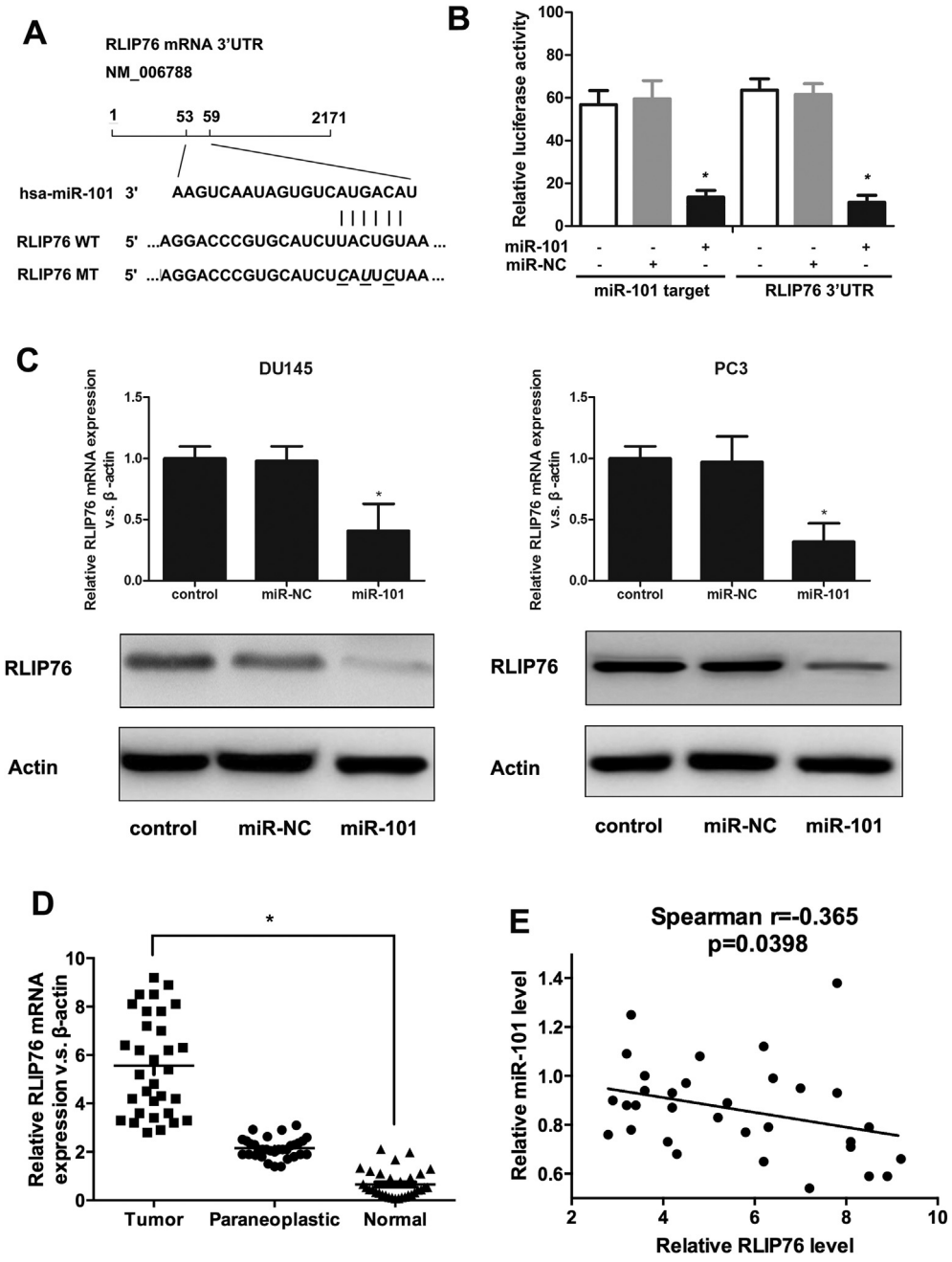

Fig. 2. (A) Sequence of wild type and mutant miR-101 target sites in the RLIP76. (B) Luciferase reporter assay in DU145 cells. *$P < 0.05$ (C) qRT-PCR demonstrates that PLIP76 mRNA was significantly suppressed in DU145 and PC3 cells expressing miR-101 compared to control and miR-NC cells. Western-blot analysis revealed that miR-101 specifically repressed PLIP76 protein expressions. (D) qRT-PCR revealed the miRNA levels of RLIP76 in human prostate cancer, paraneoplastic and normal groups. *$P < 0.05$. (E) Correlation between RLIP76 mRNA expression and miR-101 expression.

101, miR-NC or control. As illustrated in Fig. 4A, miR-101 significantly decreased phosphorylation of both PI3K and Akt protein expressions in DU145 and PC3 cells. Meantime, no difference in total PI3K and Akt was detected among miR-101, miR-NC or control transfected cells. Moreover, the effect of miR-101 on phosphorylation of Akt and phosphorylation of PI3K was abrogated by RLIP76 transfection. In addition, RLIP76 overexpression led to activation of p-PI3K and p-Akt in DU145 and PC3 cells, which is consistent with previous report (Fig. 4A). A PI3K inhibitor, LY294002, increased cell apoptosis to a similar extent as miR-101 in a dose-dependent manner in both cell lines (Fig. 4B). These results indicate that miR-101 down-regulation of RLIP76 results in the suppression of phosphorylation of PI3K and Akt followed by an induction of apoptosis.

3.6. Bcl-2 is inhibited by miR-101 through RLIP76

Considering the increased rate of apoptosis in miR-101-transfected cells, we analyzed Bcl-2 expression levels in mock, miR-NC, and miR-101-transfected DU145 cells. As shown in Fig. 4C, miR-101 resulted in a significant decrease in Bcl-2 protein expression in DU145 cells transfected with miR-101. The results of RT-PCR confirmed the similar results (Fig. 4C). Similar data were obtained with PC3 cells. In addition, when RLIP76 was transfected into both

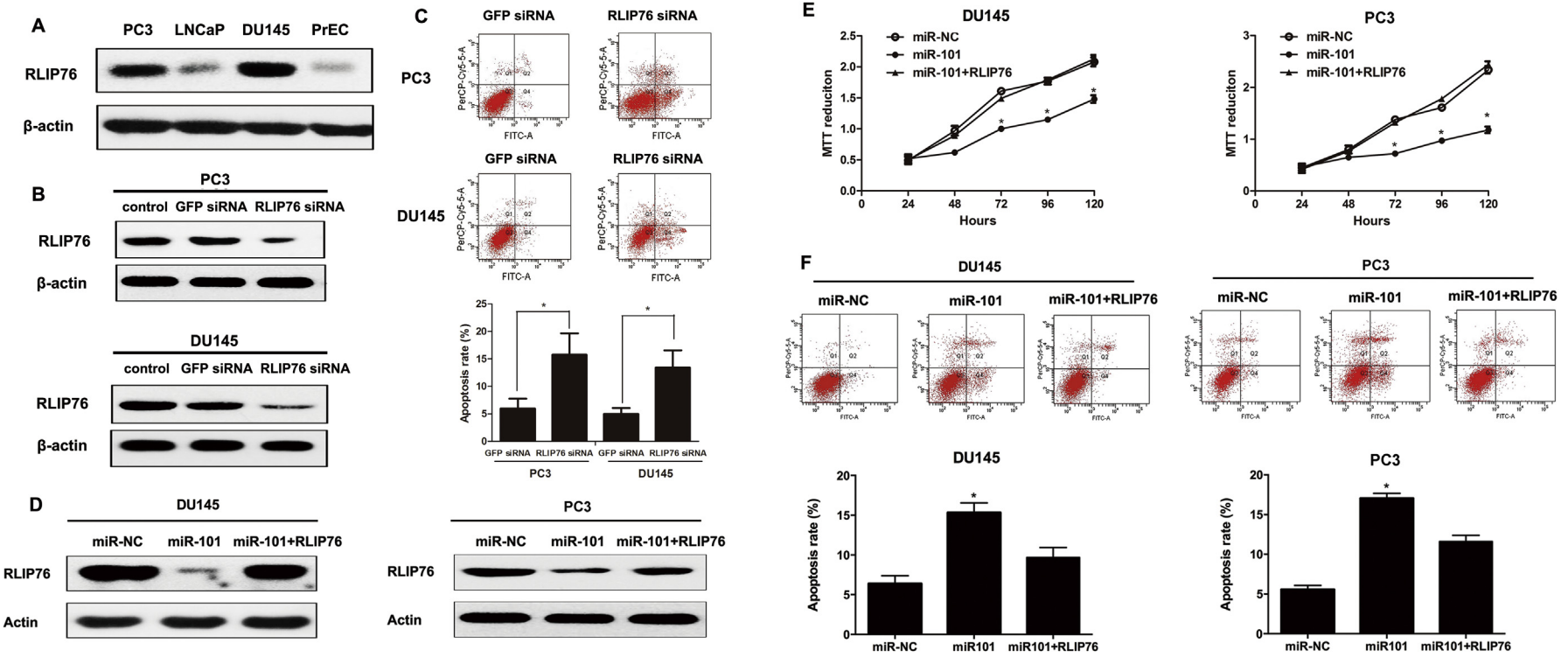

Fig. 3. (A) Western blot analysis shows the results of RLIP76 expression in PC3, DU145, LNCaP, and PrEC. (B) RLIP76 expression was significantly decreased in DU145 and PC3 cells transfected with RLIP76 siRNA compared to cells transfected with GFP siRNA. $^{*}P < 0.01$ (C) The number of apoptotic cells was significantly higher in DU145 and PC3 cells transfected with RLIP76-targeted siRNA as measured by PI staining and flow cytometry. $^{*}P < 0.05$ (D) Western blot analysis shows the results of RLIP76 expression in miR-101 transfected cells after up-regulation of RLIP76. (E) MTT assay of DU145 and PC3 cells co-transfected with miR-101 and RLIP76 or the control. $^{*}P < 0.05$ (D) Apoptosis assay of DU145 and PC3 cells co-transfected with miR-101 and RLIP76 or the control. $^{*}P < 0.05$.

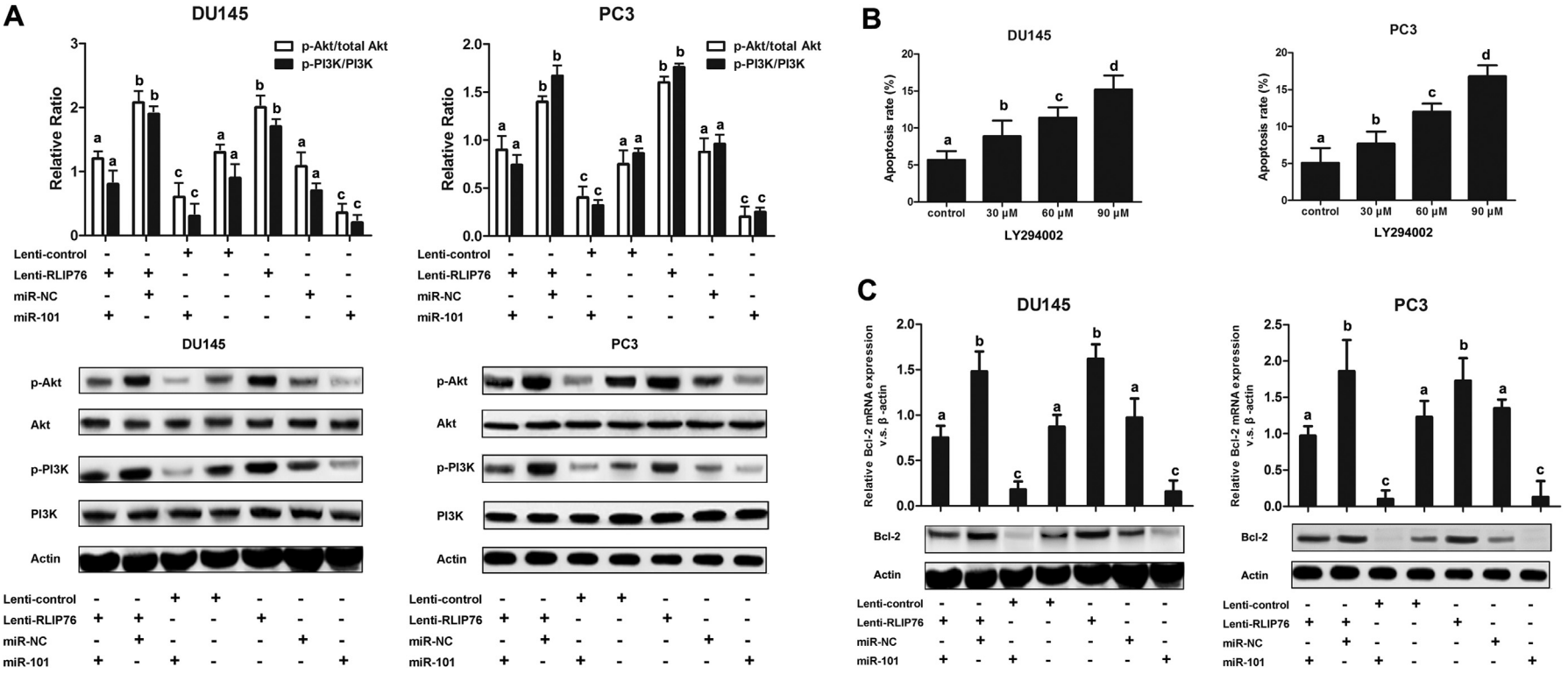

Fig. 4. (A) RLIP76 overexpression blocks the effect of miR-101 on phosphorylations of PI3K/Akt without affecting total PI3K and Akt expression. Values representing the mean ± SD (n = 3) with different letters are significantly different. $^{*}P < 0.05$ (B) PC3 and DU145 cells were treated with a PI3K inhibitor, LY294002, at the indicated concentration for 48 h. Values representing the mean ± SD (n = 3) with different letters are significantly different. $^{*}P < 0.05$ (C) miR-101 suppresses Bcl-2 protein expression through RLIP76. Values representing the mean ± SD (n = 3) with different letters are significantly different. $^{*}P < 0.05$.

cell liens (DU145 and PC3) to enhance RLIP76 expression, inhibition of Bcl-2 by miR-101 was blocked (Fig. 4C), thus suggesting a modulating role of RLIP76 in miR-101 inhibition of Bcl-2.

4. Discussion

Recent studies suggested that miR-101 could serve the function of a tumor suppressor gene in various cancers [10,17]. Furthermore, Varambally's work demonstrates that miR-101 expression was somatically lost in 37.5% of clinically localized prostate cancer cells [12]. These findings promoted us to explore its biological functions in prostate cancers. In this study, we confirmed that the levels of miR-101 expression directly influenced the apoptosis of prostate cancer cells in vitro, consistent with previous findings showing that miR-101 is related with lower proliferation in malignant prostate cancers [12]. Our data also support previous results indicating that miR-101 can promote cell apoptosis of a wide variety of tumors, including squamous cell and hepatocellular carcinoma [19,20]. To investigate the mechanisms of apoptosis induced by miR-101, we used target scan to identify the potential target of miR-101. We showed that RLIP76 is a novel target of miR-101 in prostate cancers. The miR-101 treatment resulted in a significant decrease in the levels of PI3K/Akt phosphorylation and Bcl-2 protein expression, which was restored by upregulation of RLIP76 expression. In addition, we demonstrated the anti-tumor effect of miR-101 in DU145 and PC3 cells was significantly counteracted by treatment with RLIP76 overexpression. Our data also supported that RLIP76 was critical in the regulation of PI3K and Akt activity and Bcl-2 expression in prostate cancer cells. The upregulation of RLIP76 expression significantly increased the phosphorylation of PI3K, Akt and Bcl-2. Considering above results, we strongly suggest that the markedly enhanced prostate cancer cell apoptosis by miR-101 could be attributed to RLIP76-dependent suppression of PI3K/AKT/Bcl-2 pathway in prostate cancers.

RLIP76 plays a key role in pathological progression of cancers, including cell proliferation, apoptosis and invasion. Importantly, it has been reported that RLIP76 deletion contributes to the regression of prostate cancer xenografts [18]. In addition, RLIP76 produces oncogenic action by enhancing PI3K-AKT signaling in human cancers [21]. To address the molecular mechanisms of miR-101-mediated cell proliferation, we further investigated RLIP76, PI3K and AKT expressions and found that miR-101 could regulate PI3K-AKT signaling by targeting RLIP76. These results are similar to the previous study that knockdown of RLIP76 expression can significantly suppress PI3K-AKT pathway in pancreatic cancer [21]. The suppression of PI3K-AKT pathway may be caused by the decrease in RLIP76 expression when miR-101 is overexpressed. Thus, our study provides experimental evidence that miR-101 may be probably involved in suppressing oncogenesis for prostate cancer through RLIP76-induced PI3K-AKT signaling.

Apoptosis, a genetically encoded program of cells death, plays a critical role in the prostate cancer progression. Emerging evidences indicated that Bcl-2, an important member of Bcl-2 family group, functions as an anti-apoptotic marker in various cancers. Bcl-2 family induced apoptosis pathway is the major apoptosis pathway leading to caspase activation and cell death [22]. It is also noteworthy that several meta-analyses have proved the favorable prognostic values of Bcl-2 expression in patients with prostrate cancer [23,24]. Authors believed that Bcl-2 positive tumors had a significantly better survival than those with Bcl-2 negative tumors. In agreement with these notions, we investigated the expression of Bcl-2 after miR-101 transfection to clarify the mechanisms of apoptosis in prostate cancer. We showed that miR-101 altered the expression of Bcl-2 on both mRNA and protein levels, implying a functional interaction between miR-101 and the intrinsic apoptotic pathways.

In conclusion, this work indicate that a potential mechanism by which miR-101 induces apoptosis in prostate cancer cells is through decreasing RLIP76 expression followed by concomitant suppressions of PI3K/AKT/Bcl-2 pathway. We believe that miR-101 may be a promising therapeutic target for treating prostate cancers.

Disclosure statement

The authors declare no conflict of interest.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.032.

References

[1] I.F. Tannock, et al., Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer, N. Engl. J. Med. 351 (15) (2004) 1502–1512.
[2] J.B. Aragon-Ching, The evolution of prostate cancer therapy: targeting the androgen receptor, Front. Oncol. 4 (2014) 295.
[3] S. Giacinti, et al., Resistance to abiraterone in castration-resistant prostate cancer: a review of the literature, Anticancer Res. 34 (11) (2014) 6265–6269.
[4] R. Kalla, et al., MicroRNAs: new players in IBD, Gut 64 (3) (2015 Mar) 504–517.
[5] G. Cheng, Circulating miRNAs: roles in cancer diagnosis, prognosis and therapy, Adv. Drug Deliv. Rev. 81C (2015) 75–93.
[6] Y.W. Kong, et al., microRNAs in cancer management, Lancet Oncol. 13 (6) (2012) e249–e258.
[7] X. Wen, F.M. Deng, J. Wang, MicroRNAs as predictive biomarkers and therapeutic targets in prostate cancer, Am. J. Clin. Exp. Urol. 2 (3) (2014) 219–230.
[8] J.M. Friedman, et al., The putative tumor suppressor microRNA-101 modulates the cancer epigenome by repressing the polycomb group protein EZH2, Cancer Res. 69 (6) (2009) 2623–2629.
[9] A. Strillacci, et al., MiR-101 downregulation is involved in cyclooxygenase-2 overexpression in human colon cancer cells, Exp. Cell. Res. 315 (8) (2009) 1439–1447.
[10] J.G. Zhang, et al., MicroRNA-101 exerts tumor-suppressive functions in non-small cell lung cancer through directly targeting enhancer of zeste homolog 2, J. Thorac. Oncol. 6 (4) (2011) 671–678.
[11] P. Cao, et al., MicroRNA-101 negatively regulates Ezh2 and its expression is modulated by androgen receptor and HIF-1alpha/HIF-1beta, Mol. Cancer 9 (2010) 108.
[12] S. Varambally, et al., Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer, Science 322 (5908) (2008) 1695–1699.
[13] Q. Wang, et al., RLIP76 is overexpressed in human glioblastomas and is required for proliferation, tumorigenesis and suppression of apoptosis, Carcinogenesis 34 (4) (2013) 916–926.
[14] Q. Wang, et al., Knockdown of RLIP76 expression by RNA interference inhibits invasion, induces cell cycle arrest, and increases chemosensitivity to the anticancer drug temozolomide in glioma cells, J. Neurooncol 112 (1) (2013) 73–82.
[15] S. Awasthi, et al., RLIP76 and Cancer, Clin. Cancer Res. 14 (14) (2008) 4372–4377.
[16] R. Vatsyayan, et al., RLIP76: a versatile transporter and an emerging target for cancer therapy, Biochem. Pharmacol. 79 (12) (2010) 1699–1705.
[17] Q. Bu, et al., Enforced expression of miR-101 enhances cisplatin sensitivity in human bladder cancer cells by modulating the cyclooxygenase-2 pathway, Mol. Med. Rep. 10 (4) (2014) 2203–2209.
[18] S.S. Singhal, et al., Regression of prostate cancer xenografts by RLIP76 depletion, Biochem. Pharmacol. 77 (6) (2009) 1074–1083.
[19] Y. Xu, et al., miR-101 inhibits autophagy and enhances cisplatin-induced apoptosis in hepatocellular carcinoma cells, Oncol. Rep. 29 (5) (2013) 2019–2024.
[20] R. Banerjee, et al., The tumor suppressor gene rap1GAP is silenced by miR-101-mediated EZH2 overexpression in invasive squamous cell carcinoma, Oncogene 30 (42) (2011) 4339–4349.
[21] K. Leake, et al., RLIP76 regulates PI3K/Akt signaling and chemo-radiotherapy resistance in pancreatic cancer, PLoS One 7 (4) (2012) e34582.
[22] V. Nikoletopoulou, et al., Crosstalk between apoptosis, necrosis and autophagy, Biochim. Biophys. Acta 1833 (12) (2013) 3448–3459.
[23] S. Baspinar, et al., The relation of beclin 1 and bcl-2 expressions in high grade prostatic intraepithelial neoplasia and prostate adenocarcinoma: a tissue microarray study, Pathol. Res. Pract. 210 (7) (2014) 412–418.
[24] L. Zhao, et al., Tissue biomarkers for prognosis of prostate cancer: a systematic review and meta-analysis, Cancer Epidemiol. Biomarkers Prev. 23 (6) (2014) 1047–1054.