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Downregulation of miR-485-3p promotes proliferation, migration and invasion in prostate cancer through activation of TGF-β signaling

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Summary. Background. Prostate cancer (PC) is the second leading cause of cancer-related death among men worldwide. Downregulation of miR-485-3p has been revealed to participate in the tumorigenesis and progression of many types of cancer. However, the clinical and biological role of miR-485-3p in PC remains largely unknown.

Methods. The expression of miR-485-3p was analyzed in the published databases and detected in our clinical samples and cell lines by RT-qPCR assay. CCK8, transwell invasion and migration, and colony formation assays were performed to investigate the biological function of miR-485-3p. Bioinformatical analysis, RIP, western blotting and luciferase reporter assays were carried out to explore the downstream mechanism of miR-485-3p.

Results. The level of miR-485-3p was downregulated in PC tissues, particularly in primary PC tissues with metastasis relative to normal prostate tissues. miR-485-3p downregulation was positively correlated with poor disease-free and overall survival in patients with PC. Functionally, miR-485-3p overexpression dramatically suppressed the proliferation, migration and invasion ability of PC cells in vitro. Mechanistically, miR-485-3p overexpression suppressed the activity of TGF-β signaling by targeting TGFBR2 to play tumor-suppressive roles in PC progression.

Conclusion. Our study reports the miR-485-3p/TGFBR2/ TGF-β signaling axis in tumor development of PC, suggesting miR-485-3p may be a potential target to develop therapeutic strategies against PC.

Key words: miR-485-3p, Prostate cancer, Proliferation, Migration, Invasion, TGFBR2, TGF-β signaling

Introduction

Prostate cancer (PC) is one of the most commonly diagnosed cancers and ranks second in terms of cancer-related death among men worldwide (Dai et al., 2019; Lang et al., 2020). With the development of imaging technology and diagnostic technology, PC patients can be diagnosed and treated at an early stage. Treatments for inhibition of the androgen receptor pathway is the main therapeutic method for patients with PC and most patients are sensitive to androgen-deprivation therapy (Coleman et al., 2020; Lang et al., 2021). However, most patients will be resistant to androgen-deprivation therapy and progress to the stage of castration-resistant PC, which is considered to be one lethal subtype of PC and the treatment options are limited. Therefore, understanding the precise mechanism of PC initiation and progression will contribute to the development of therapeutic strategies against PC.

miRNAs are a class of RNA transcripts with 18-25 nt, which have no protein-coding ability and interact with the 3'UTR of target mRNA to decrease their expression (Chen et al., 2021; Li et al., 2021; Yin et al., 2021). miRNAs have been documented in various

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Abbreviations. PC, prostate cancer; RT-qPCR, Real-time quantitative PCR; ANT, adjacent normal tissues; TCGA, The Cancer Genome Atlas; GSEA, gene set enrichment analysis.

biological and pathological processes, such as tumor growth and metastasis in human cancers (Jiang et al., 2021; Wang et al., 2021). Dysregulated expression of miRNA has been reported to play important roles in PC progression and be related to poor survival of patients (Zhang et al., 2020a,b; Zhou et al., 2020; Jiang et al., 2021). Downregulation of miR-92a significantly promoted cell viability and invasiveness of PC-3 cells by targeting transcriptional factor, SOX4 (Liao et al., 2020). miR-16-5p took part in regulating cell cycle, cell growth and apoptosis in PC cells via degrading mRNA of AKT3 (Wang et al., 2020). Downregulation of p27 protein mediated by miR-196a enhanced PC cell proliferation and predicted biochemical recurrence of patients (Zhan et al., 2020). miR-485-3p has been revealed to play tumor-promoting or -suppressing roles in multiple cancers (Lou et al., 2016; Du et al., 2018; Taherdangkoo et al., 2020). Nevertheless, the biological function of miR-485-3p in PC is still unclear.

In this study, it was found that the expression of miR-485-3p was reduced in metastatic and primary PC tissues relative to normal prostate tissues. miR-485-3p downregulation was related to poor disease-free and overall survival of patients with PC. Functionally, miR-485-3p overexpression markedly decreased the ability of proliferation, invasion and migration in PC cells. Mechanistically, miR-485-3p suppressed TGF-β signaling via inhibition of TGFBR2.

Materials and methods

Cell culture and clinical samples

Human cell lines (RWPE-1, LNCaP, 22RV1, DU145, C4-2B, PC-3 and VCaP) were used in this study. Cell culture was performed according to the description in the previous study (Dai et al., 2019). All cell lines were incubated in the recommended medium. Human samples, including the tissues of PC and normal prostate, were collected from our hospital. Informed consent was obtained from all patients. This study was approved by the ethical committee of The First Affiliated Hospital of Shandong First Medical University (approval-No. 2021S-959).

Real-time quantitative PCR (RT-qPCR)

RNA extraction was performed using an EN-press RNA Purification Kit. The detailed process of RT-qPCR was described in a previous study (Xiong et al., 2018). The primers used in this study were as follows: TGFBR2, 5'-GTCTGTGGATGACCTGGCTAAC-3' and 5'-GACATCGGTCTGCTTGAAGGAC-3', GAPDH, 5'-GTCTCCTCTGACTTCAACAGCG-3' and 5'-ACCACCCTGTTGCTGTAGCCAA-3'.

Western blotting

Western blotting was carried out as previously described (Xia et al., 2021). Antibodies against TGFBR2, SMAD3 and p-SMAD3 were purchased from Proteintech. Anti-GAPDH (Proteintech) antibodies were used as the loading control.

Cell proliferation assay

CCK8 and colony formation and assays were used to determine cell proliferation ability and conducted according to a previous study (Hong et al., 2021; Jia et al., 2021).

Transwell assay

Transwell assay was carried out to detect the ability of invasion and migration of PC cells. The detailed information is as described previously (Zhao et al., 2018).

Luciferase reporter assay

TGFBR2-wt plasmid or TGFBR2-mut plasmid and miR-485-3p plasmid were transfected into PC cells using Lipofectamine 3000. After 48h of transfection, Dual-luciferase activity reporter assay kit (Promega) was used to determine luciferase activity normalized to Renilla luciferase activity.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 8. All data were reported as mean ± standard deviation (SD). The group difference was analyzed by Student t-test and Mann-Whitney U test. $P<0.05$ was considered statistically significant.

Results

The expression of miR-485-3p is decreased in PC tissues and cell lines

miR-485-3p was dysregulated and played significant roles in multiple human cancers. Nevertheless, the clinical and biological function of miR-485-3p in PC progression were still unclear. Therefore, the expression pattern of miR-485-3p in PC tissues and adjacent normal tissues (ANT) from the dataset of GSE21306 was analyzed. We found that the expression level of miR-485-3p was decreased from ANT and primary PC (p-PC) to metastatic PC (m-PC) (Fig. 1A). RT-qPCR analysis in our clinical samples indicated that miR-485-3p level was prominently increased in paired ANT compared with PC (Fig. 1B). Meanwhile, we found that decreased miR-485-3p expression was prevalent in PC tissues with metastasis relative to ANT and PC tissues without metastasis (Fig. 1C). Further RT-qPCR analysis was performed to detect miR-485-3p level in cell lines of PC. The results indicated that miR-485-3p level was significantly reduced in PC cell lines relative to normal prostate cells (Fig. 1D). Survival analysis based on TCGA datasets demonstrated that the decreased

expression of miR-485-3p was positively correlated with shorter overall and disease-free survival (Fig. 1E,F). Collectively, miR-485-3p expression is reduced in PC and predicts poor prognosis in PC patients.

Overexpression of miR-485-3p inhibits proliferation, migration and invasion in PC

In order to investigate the function of miR-485-3p in PC, GSEA using the data from TCGA was performed. We found that reduced miR-485-3p expression was correlated with proliferation- and metastasis-relevant gene sets (Fig. 2A). Then, miR-485-3p was overexpressed in PC cells with lentivirus infection (Fig. 2B). CCK8 and colony formation assays were conducted to determine the proliferation capability of PC cells and we found that miR-485-3p overexpression significantly inhibited cell viability shown by CCK8 and colony formation ability indicated by colony formation assay, which demonstrated that miR-485-3p inhibited the proliferation ability of PC cells (Fig. 2C-E). The results from Transwell assays indicated that overexpression of miR-485-3p markedly suppressed the migration and invasion capability in PC cells (Fig. 2F). Taken together, miR-485-3p inhibits PC cell proliferation, migration and invasion.

miR-485-3p inhibits the activity of TGF-β signaling

Previous studies revealed that signaling pathways may play crucial roles in the microRNA-mediated tumor progression and GSEA results suggested that the TGF-β pathway may mediate the role of miR-485-3p in PC (Fig. 1A,B). Luciferase reporter assay indicated that miR-485-3p overexpression prominently reduced the luciferase activity of TGF-β signaling in PC cells (Fig. 3C). Western blotting assay indicated that miR-485-3p overexpression dramatically reduced the activity of the TGF-β pathway (Fig. 3D). Further analysis demonstrated that the activation of TGF-β signaling using SMAD3 mutants (Cai et al., 2017) reversed the inhibitory effect induced by miR-485-3p overexpression on PC cell proliferation and invasiveness (Fig. 3E,F). The above results demonstrate that miR-485-3p inhibits PC progression through the inactivation of TGF-β signaling.

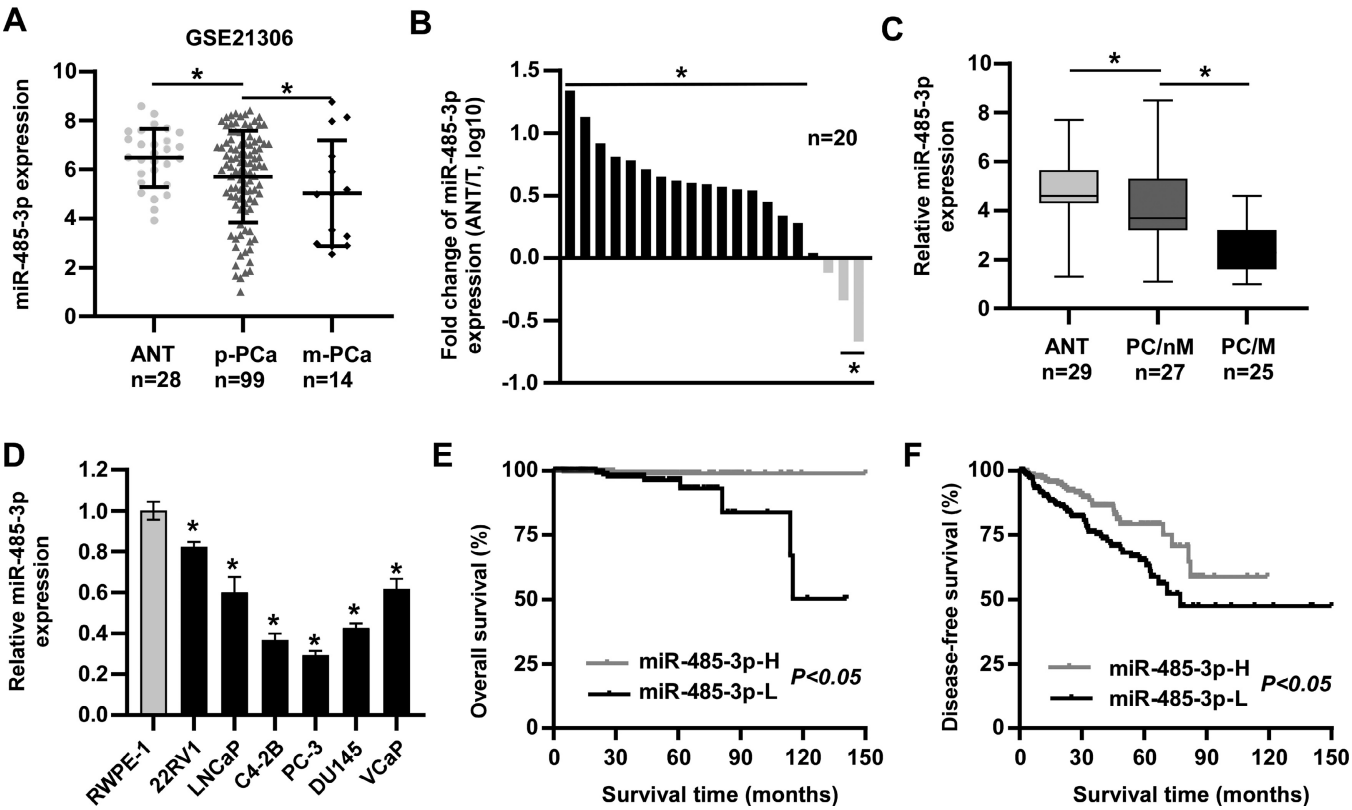

Fig. 1. miR-485-3p expression is decreased in PC tissues and cell lines. **A.** miR-485-3p expression in ANT (n=8), primary PC tissues (p-PC, n=99), and metastatic PC tissues (m-PC, n=14) in GEO dataset (GSE21036). **B.** Real-time PCR analysis of the fold change of miR-485-3p expression in 20 paired PC tissues and their matched ANT. **C.** Real-time PCR analysis of miR-485-3p expression in ANT (n=29), PC without metastasis (PC/nM, n=27), and PC with metastasis (PC/M, n=25). **D.** Real-time PCR analysis of miR-485-3p expression levels in different PC cell lines. **E, F.** Kaplan-Meier analysis of overall survival **(E)** and disease-free survival **(F)** curves of the PC patients stratified by miR-485-3p expression in TCGA dataset. *P<0.05.

miR-485-3p targets TGFBR2 to inactivate TGF-β signaling

To detect the target of miR-485-3p, we analyzed the public databases, StarBase and mirDIP, and found that TGFBR2 may be the target of miR-485-3p, which was one key regulator of the TGF-β pathway (Fig. 4A). RT-qPCR and western blotting assays were performed to verify the above prediction and the results indicated that miR-485-3p can downregulate TGFBR2 expression at both mRNA and protein level in PC cells (Fig. 4B,C). One direct binding site between TGFBR2 and miR-485-3p was indicated by StarBase and the mutated miR-485-3p was designed (Fig. 4D). Further RIP experiments indicated that overexpression of miR-485-3p dramatically increased the enrichment of TGFBR2 on Ago2 and the mutated miR-485-3p did not affect the enrichment of TGFBR2 on Ago2 (Fig. 4E). Meanwhile, we found that miR-485-3p overexpression dramatically reduced 3'-UTR's luciferase activity of TGFBR2, whereas the mutated miR-485-3p had no similar effect (Fig. 4F). The above findings suggested the direct

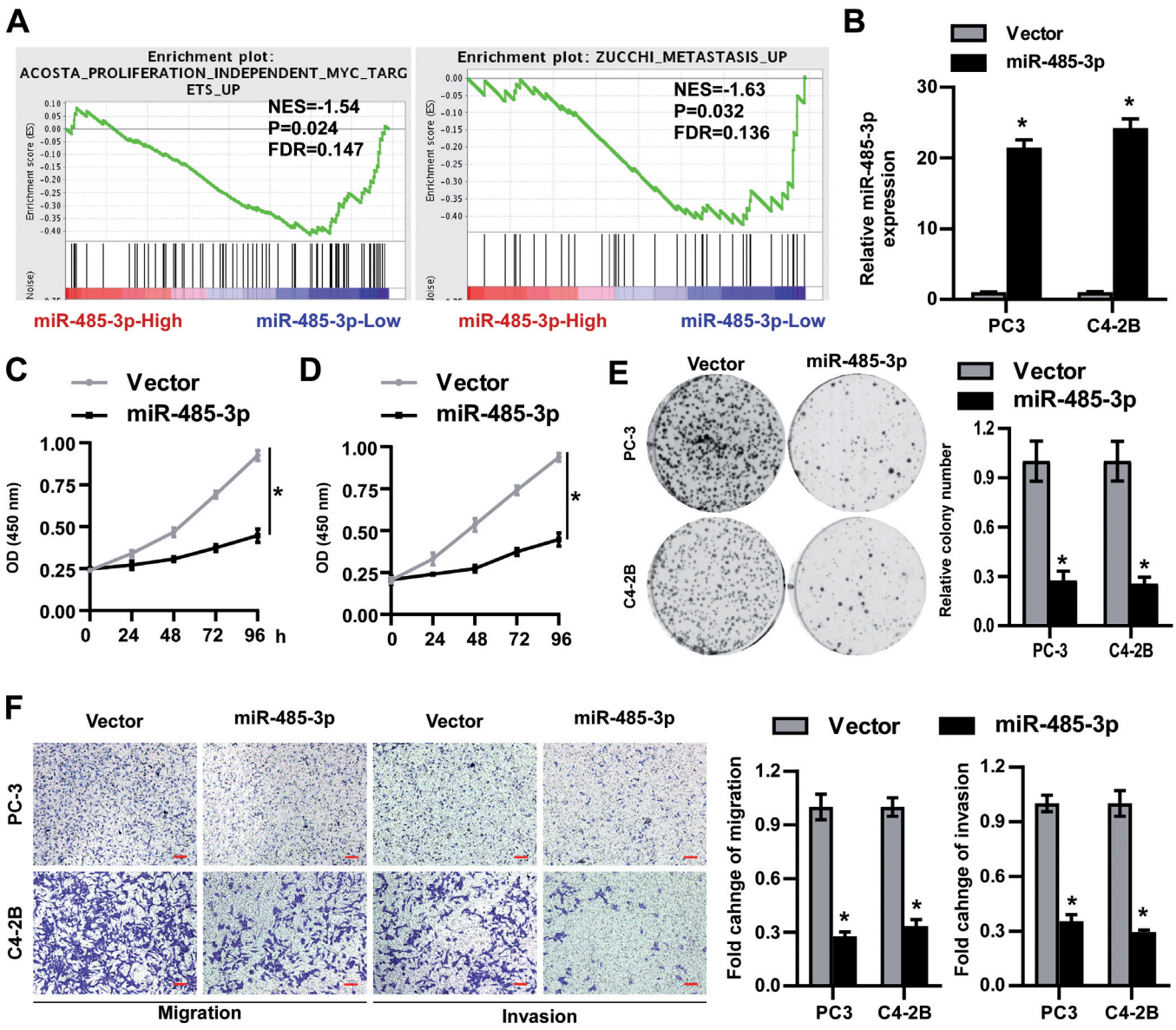

Fig. 2. Overexpression of miR-485-3p inhibits proliferation, migration and invasion in PC. **A.** Gene set enrichment analysis (GSEA) revealed that miR-485-3p downregulation significantly and positively correlated with the proliferation- and metastasis-related gene signature. **B.** miR-485-3p expression in the indicated groups. *P<0.05. **C-E.** CCK8 **(C, D)** and colony formation assays **(E)** were conducted to test cell proliferation in PC-3 and C4-2B cells. **F.** Upregulating miR-485-3p inhibited invasion and migration abilities in PC cells. *P<0.05. Scare bars: 100 um.

binding between miR-485-3p and TGFBR2. To further investigate whether TGFBR2 mediated the inactivation of TGF-β signaling induced by miR-485-3p, we overexpressed TGFBR2 in PC cells (Fig. 4G). The results from Fig. 4H, overexpression of TGFBR2 abolished the inhibitory function of miR-485-3p on the activity of the TGF-β pathway and the mutated miR-485-3p had no effect on the TGF-β pathway. These results suggest that miR-485-3p leads to the inactivation of the TGF-β pathway by targeting TGFBR2.

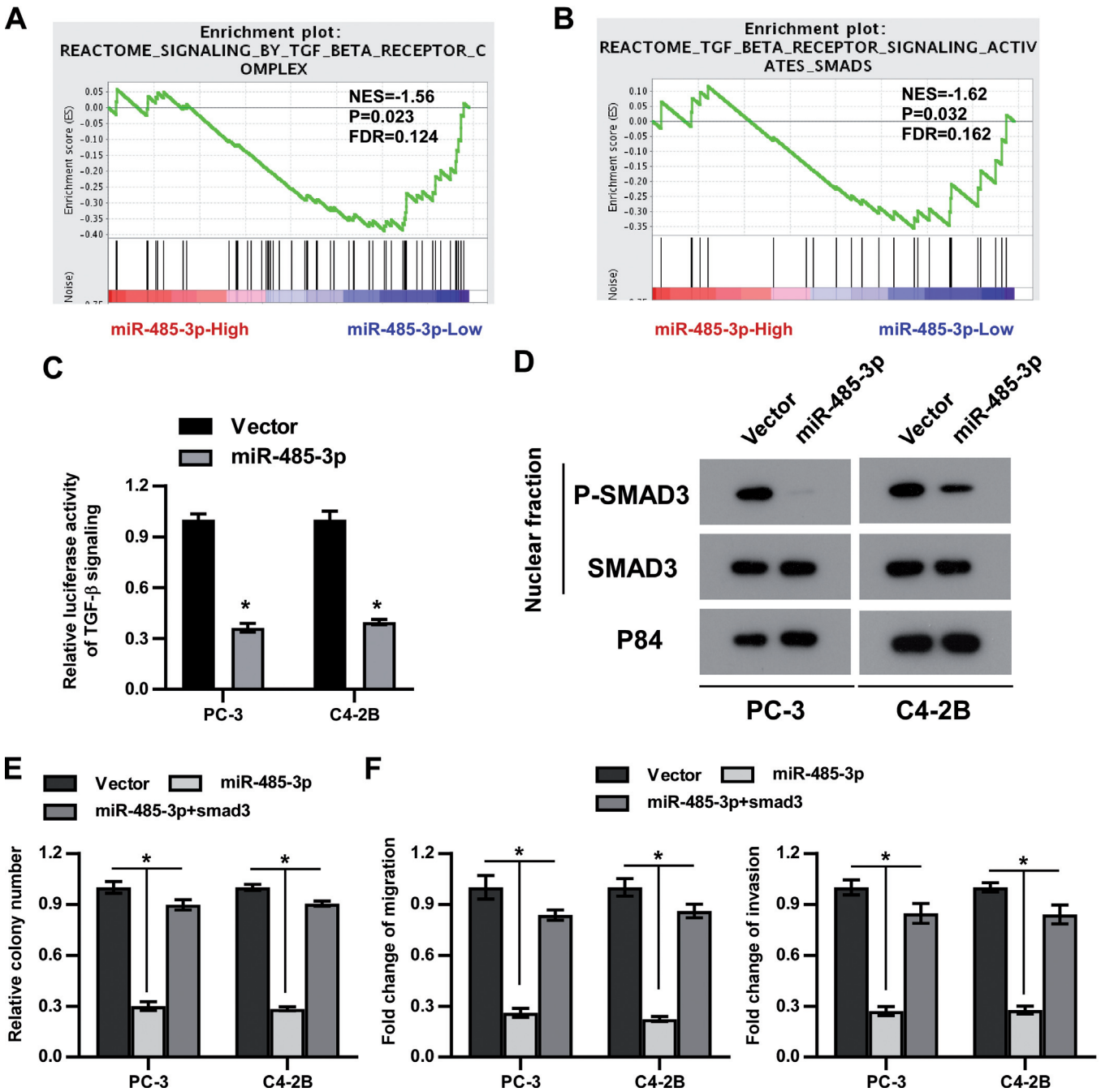

Fig. 3. miR-485-3p suppresses the activity of TGF-β signaling. **A, B.** Gene set enrichment analysis (GSEA) revealed that miR-485-3p downregulation was significantly and positively correlated with the TGF-β signaling. **C.** Relative luciferase activities of different pathways in the indicated groups. **D.** Western blot assay showed the p-samd3 and smad3 proteins expression in the indicated groups. **E.** Colony formation assay was conducted to test cell proliferation in the indicated group. **F.** Transwell assay was performed to analyze migration and invasion of PC-3 and C4-2B cells in the indicated groups. *P<0.05.

Discussion

The main finding of this study is to report the tumor-suppressive role of miR-485-3p in PC. Previous mounting studies have indicated that miR-485-3p expression was dysregulated in many human cancers. In the current study, the expression of miR-485-3p was markedly reduced in PC, particularly in PC with metastasis relative to paired normal prostate tissues, and similar results were observed in PC cell lines. Survival analysis in patients with PC showed that the decreased level of miR-485-3p was correlated with poor prognosis.

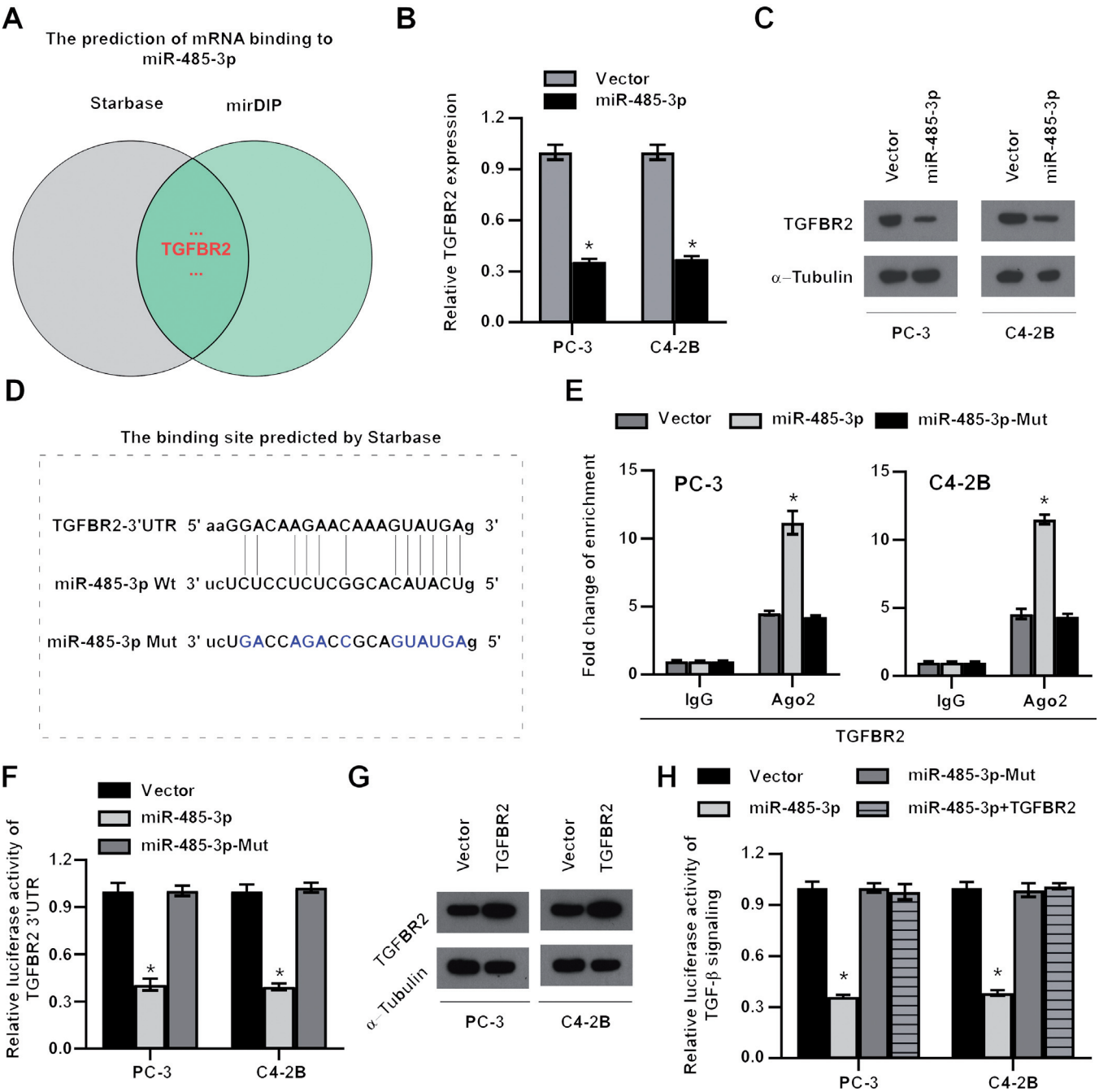

Fig. 4. TGFBR2 is the direct target of miR-485-3p. **A.** The prediction of mRNAs that may bind to miR-485-3p. **B.** Real-time PCR analysis of TGFBR2 expression in the indicated groups. **C.** Western blotting analysis of TGFBR2 expression in the indicated groups. **D.** The binding site between TGFBR2 and miR-485-3p. **E.** RIP assay showed the enrichment of TGFBR2 in Ago2 protein in the indicated groups. **F.** Luciferase reporter assay showed the luciferase activity of TGFBR2 in the indicated group. *P<0.05.

Furthermore, in vitro experiments indicated that miR-485-3p overexpression potently inhibited the abilities of proliferation, migration and invasion in PC cells. Mechanistically, the TGF-β pathway was suppressed by the overexpression of miR-485-3p and TGFBR2 was directly targeted by miR-485-3p, which mediated the regulation of TGF-β pathway induced by miR-485-3p.

microRNAs participate in the initiation and development in many human cancers (Chen et al., 2021; Li et al., 2021; Liang et al., 2021; Yin et al., 2021). miR-485-3p served as a suppressor in colorectal cancer by decreasing TPX expression and upregulating P21 level (Taherdangkoo et al., 2020). miR-485-3p and -5p expression were reduced in breast cancer and significantly suppressed the invasion and migration ability in breast cancer by sponging PGC-1α mRNA (Lou et al., 2016). For cervical cancer, miR-485-3p, which was sponged by lncRNA SNHG6, inhibited cell growth and radioresistance by downregulating the expression of STYX (Liu et al., 2020). In patients with glioblastoma, the level of miR-485-3p in serum could be used as one biomarker to predict survival (Wang et al., 2017). Therefore, the function of miR-485-3p in PC may contribute to developing novel therapeutic strategies.

TGF-β signaling has been reported to be involved in a variety of biological and pathological processes (Dai et al., 2019; Lang et al., 2020), including cancers (Dai et al., 2017). The TGF-β pathway may play dual roles in tumor progression, by inhibiting tumor initiation in early-stage and promoting tumor progression in the developing stage (Lang et al., 2020). In PC, TGF-β signaling not only enhanced cell invasion and migration, but also mediated the crosstalk between cancer cells and tumor microenvironment (Fournier et al., 2015; Chen et al., 2017; Dai et al., 2017, 2019; Huang et al., 2018; Meng et al., 2018; Wu et al., 2018). Huang et al. reported that the TGF-β pathway promoted PC cell invasion and migration and was regulated by miR-133b (Huang et al., 2018). Osteoclast-derived TGF-β activated the TGF-β pathway in PC cells, which led to the increased survival, proliferation and colonization of PC cells in bone (Fournier et al., 2015; Zhang et al., 2020a,b; Di Paolo et al., 2021). In this study, the decreased miR-485-3p expression resulted in the activation of the TGF-β pathway in PC. Further analysis indicated that TGFBR2 was directly targeted and downregulated by miR-485-3p. TGFBR2 was one crucial regulator of the TGF-β pathway and mediated the regulation between the TGF-β pathway and miR-485-3p. This study uncovers one novel regulator of the TGF-β pathway.

Conclusion

This study reveals the crucial role of miR-485-3p/TGFBR2/TGF-β signaling axis in tumor progression in PC, indicating that miR-485-3p may be a potential target to develop therapeutic strategies against PC.

Acknowledgements. Not applicable

Conflict of interest. The authors declare that there is no conflict of interest.

References

Cai J., Fang L., Huang Y., Li R., Xu X., Hu Z., Zhang L., Yang Y., Zhu X., Zhang H., Wu J., Huang Y., Li J., Zeng M., Song E., He Y., Zhang L. and Li M. (2017). Simultaneous overactivation of Wnt/beta-catenin and TGFbeta signalling by miR-128-3p confers chemoresistance-associated metastasis in NSCLC. Nat. Commun. 8, 15870.

Chen J., Zhang K., Zhi Y., Wu Y., Chen B., Bai J. and Wang X. (2021). Tumor-derived exosomal miR-19b-3p facilitates M2 macrophage polarization and exosomal LINC00273 secretion to promote lung adenocarcinoma metastasis via Hippo pathway. Clin. Transl. Med. 11, e478.

Chen W.Y., Tsai Y.C., Yeh H.L., Suau F., Jiang K.C., Shao A.N., Huang J. and Liu Y.N. (2017). Loss of SPDEF and gain of TGFBI activity after androgen deprivation therapy promote emt and bone metastasis of prostate cancer. Sci. Signal. 10 eaam6826.

Coleman R.E., Croucher P.I., Padhani A.R., Clezardin P., Chow E., Fallon M., Guise T., Colangeli S., Capanna R. and Costa L. (2020). Bone metastases. Nat. Rev. Dis. Primers. 6, 83.

Dai Y., Ren D., Yang Q., Cui Y., Guo W., Lai Y., Du H., Lin C., Li J., Song L. and Peng X. (2017). The TGF-beta signalling negative regulator PICK1 represses prostate cancer metastasis to bone. Br. J. Cancer 117, 685-694.

Dai Y.H., Wu Z.Q., Lang C.D., Zhang X., He S.F., Yang Q., Guo W., Lai Y.R., Du H., Peng X.S. and Ren D. (2019). Copy number gain of ZEB1 mediates a double-negative feedback loop with miR-33a-5p that regulates emt and bone metastasis of prostate cancer dependent on TGF-beta signaling. Theranostics 9, 6063-6079.

Di Paolo D., Pontis F., Moro M., Centonze G., Bertolini G., Milione M., Mensah M., Segale M., Petraroia I., Borzi C., Suatoni P., Brignole C., Perri P., Ponzoni M., Pastorino U., Sozzi G. and Fortunato O. (2021). Cotargeting of miR-126-3p and miR-221-3p inhibits PIK3R2 and pten, reducing lung cancer growth and metastasis by blocking AKT and CXCR4 signalling. Mol. Oncol. 15, 2969-2988.

Du K., Zhang X., Lou Z., Guo P., Zhang F., Wang B., Chen L. and Zhang C. (2018). MicroRNA485-3p negatively regulates the transcriptional co-repressor CTBP1 to control the oncogenic process in osteosarcoma cells. Int. J. Biol. Sci. 14, 1445-1456.

Fournier P.G., Juarez P., Jiang G., Clines G.A., Niewolna M., Kim H.S., Walton H.W., Peng X.H., Liu Y., Mohammad K.S., Wells C.D., Chirgwin J.M. and Guise T.A. (2015). The TGF-beta signaling regulator PMEPA1 suppresses prostate cancer metastases to bone. Cancer Cell 27, 809-821.

Hong Z., Xiang Z., Zhang P., Wu Q., Xu C., Wang X., Shi G., Hong Z. and Wu D. (2021). Histone acetyltransferase 1 upregulates androgen receptor expression to modulate crpc cell resistance to enzalutamide. Clin. Transl. Med. 11, e495.

Huang S., Wa Q., Pan J., Peng X., Ren D., Li Q., Dai Y., Yang Q., Huang Y., Zhang X., Zhou W., Yuan D., Cao J., Li Y., He P. and Tang Y. (2018). Transcriptional downregulation of miR-133b by rest promotes prostate cancer metastasis to bone via activating TGF-

beta signaling. Cell Death Dis. 9, 779.

Jia Q., Xie B., Zhao Z., Huang L., Wei G. and Ni T. (2021). Lung cancer cells expressing a shortened CDK16 3'UTR escape senescence through impaired miR-485-5p targeting. Mol. Oncol. (in press).

Jiang Y., Tao Y., Zhang X., Wei X., Li M., He X., Zhou B., Guo W., Yin H. and Cheng S. (2021). Loss of STAT5A promotes glucose metabolism and tumor growth through miRNA-23a-AKT signaling in hepatocellular carcinoma. Mol. Oncol. 15, 710-724.

Lang C., Dai Y., Wu Z., Yang Q., He S., Zhang X., Guo W., Lai Y., Du H., Wang H., Ren D. and Peng X. (2020). SMAD3/SP1 complex-mediated constitutive active loop between lncRNA PCAT7 and TGF-β signaling promotes prostate cancer bone metastasis. Mol. Oncol. 14, 808-828.

Lang C., Yin C., Lin K., Li Y., Yang Q., Wu Z., Du H., Ren D., Dai Y. and Peng X. (2021). m^6A modification of lncRNA PCAT6 promotes bone metastasis in prostate cancer through IGF2BP2-mediated IGF1r mRNA stabilization. Clin. Transl. Med. 11, e426.

Li H., Zhang N., Jiao X., Wang C., Sun W., He Y., Ren G., Huang S., Li M., Chang Y., Jin Z., Xie Q., Zhang X., Huang H. and Jin H. (2021). Downregulation of microRNA-6125 promotes colorectal cancer growth through YTHDF2-dependent recognition of n6-methyladenosine-modified GSK3β. Clin. Transl. Med. 11, e602.

Liang Y., Mao Q., Wang L., Xia W., Chen B., Wang H., Li R., Xu L., Jiang F. and Dong G. (2021). CircIMMP2l promotes esophageal squamous cell carcinoma malignant progression via CtBP1 nuclear retention dependent epigenetic modification. Clin. Transl. Med. 11, e519.

Liao G., Xiong H., Tang J., Li Y. and Liu Y. (2020). MicroRNA-92a inhibits the cell viability and metastasis of prostate cancer by targeting SOX4. Technol. Cancer Res. Treat. 19, 1533033820959354.

Liu J., Liu X. and Li R. (2020). LncRNA SNHG6 enhances the radioresistance and promotes the growth of cervical cancer cells by sponging miR-485-3p. Cancer Cell Int. 20, 424.

Lou C., Xiao M., Cheng S., Lu X., Jia S., Ren Y. and Li Z. (2016). MiR-485-3p and miR-485-5p suppress breast cancer cell metastasis by inhibiting PGC-1α expression. Cell Death Dis. 7, e2159.

Meng X., Vander Ark A., Daft P., Woodford E., Wang J., Madaj Z. and Li X. (2018). Loss of TGF-beta signaling in osteoblasts increases basic-FGF and promotes prostate cancer bone metastasis. Cancer Let. 418, 109-118.

Taherdangkoo K., Kazemi Nezhad S.R., Hajjari M.R. and Tahmasebi Birgani M. (2020). MiR-485-3p suppresses colorectal cancer via targeting TPX2. Bratisl. Lek. Listy. 121, 302-307.

Wang Z.Q., Zhang M.Y., Deng M.L., Weng N.Q., Wang H.Y. and Wu S.X. (2017). Low serum level of miR-485-3p predicts poor survival in patients with glioblastoma. PLoS One 12, e0184969.

Wang F., Wang W., Lu L., Xie Y., Yan J., Chen Y., Di C., Gan L., Si J., Zhang H. and Mao A. (2020). MicroRNA 16 5p regulates cell survival, cell cycle and apoptosis by targeting AKT3 in prostate cancer cells. Oncol. Rep. 44, 1282-1292.

Wang X., Qian T., Bao S., Zhao H., Chen H., Xing Z., Li Y., Zhang M., Meng X., Wang C., Wang J., Gao H., Liu J., Zhou M. and Wang X. (2021). Circulating exosomal miR-363-5p inhibits lymph node metastasis by downregulating PDGFB and serves as a potential noninvasive biomarker for breast cancer. Mol. Oncol. 15, 2466-2479.

Wu J., Sun H., Li J., Guo Y., Zhang K., Lang C., Zou C. and Ma H. (2018). Increased survival of patients aged 0-29 years with osteosarcoma: A period analysis, 1984-2013. Cancer Med. 7, 3652-3661.

Xia R.M., Liu T., Li W.G. and Xu X.Q. (2021). RNA-binding protein RBM24 represses colorectal tumourigenesis by stabilising PTEN mRNA. Clin. Transl. Med. 11, e383.

Xiong Z., Ye L., Zhenyu H., Li F., Xiong Y., Lin C., Wu X., Deng G., Shi W., Song L., Yuan Z. and Wang X. (2018). ANP32E induces tumorigenesis of triple-negative breast cancer cells by upregulating E2F1. Mol. Oncol. 12, 896-912.

Yin D., Hu Z.Q., Luo C.B., Wang X.Y., Xin H.Y., Sun R.Q., Wang P.C., Li J., Fan J., Zhou Z.J., Zhou J. and Zhou S.L. (2021). Linc01133 promotes hepatocellular carcinoma progression by sponging miR-199a-5p and activating annexin A2. Clin. Transl. Med. 11, e409.

Zhan B., Huang L., Chen Y., Ye W., Li J., Chen J., Yang S. and Jiang W. (2020). MiR-196a-mediated downregulation of $p27^{Kip1}$ protein promotes prostate cancer proliferation and relates to biochemical recurrence after radical prostatectomy. Prostate 80, 1024-1037.

Zhang J., Jiang M., Qian L., Lin X., Song W., Gao Y. and Zhou Y. (2020a). The STAT3-miR-223-TGFBR3/HMGCS1 axis modulates the progression of cervical carcinoma. Mol. Oncol. 14, 2313-2331.

Zhang X., Bai J., Yin H., Long L., Zheng Z., Wang Q., Chen F., Yu X. and Zhou Y. (2020b). Exosomal miR-1255b-5p targets human telomerase reverse transcriptase in colorectal cancer cells to suppress epithelial-to-mesenchymal transition. Mol. Oncol. 14, 2589-2608.

Zhao Y., Ma J., Fan Y., Wang Z., Tian R., Ji W., Zhang F. and Niu R. (2018). TGF-beta transactivates EGFR and facilitates breast cancer migration and invasion through canonical SMAD3 and ERK/SP1 signaling pathways. Mol. Oncol. 12, 305-321.

Zhou Q., Guo J., Huang W., Yu X., Xu C. and Long X. (2020). Linc-ROR promotes the progression of breast cancer and decreases the sensitivity to rapamycin through miR-194-3p targeting MECP2. Mol. Oncol. 14, 2231-2250.

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