Regulation of *RUNX3* expression by DNA methylation in prostate cancer

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Abstract

Background: Runt-related transcription factor 3 (RUNX3) is a developmental regulator, and methylation of the RUNX3 is significantly associated with the occurrence and development of carcinogenesis. Previous studies have identified an association of increased methylation level of RUNX3 in prostate cancer (PCa); however, the role and molecular mechanism underlying aberrant methylation of the RUNX3 gene in prostate tumorigenesis remain elusive. In this study, we will investigate the role of RUNX3 promoter methylation and its gene expression in PCa cells.

Methods: The methylation of the RUNX3 in the promoter region in PCa cells was detected by bisulfite-sequencing PCR (BSP). Following treatment of the PCa cells with DNA methylation transferase inhibitor 5-AZA-2'-deoxycytidine (AZA), the effect on methylation level and expression of RUNX3 were analyzed by qRT-PCR, Western blot, and BSP assays. Furthermore, we investigated the effect of the demethylated RUNX3 on proliferation, cell cycle and apoptosis of PCa cells using CCK-8 and flow cytometry assays. Using the DNA methylation transferase (DNMT3b) knockout or overexpression models, the relationship between DNMT3b and RUNX3 methylation was further assessed by qRT-PCR, Western blot and methylation-specific PCR (MSP).

Results: The results indicated that the methylation level of RUNX3 in PCa cell lines was significantly higher than that of normal prostate epithelial (RWPE-1) cells. Furthermore, treatment with AZA not only promoted the demethylation of RUNX3 but also restored the mRNA and protein expression of RUNX3, and the reactivation of expression of the later exhibited its anti-tumor effects through regulation of the cycle progression in PCa cells. Moreover, DNMT3b could regulate the expression level of RUNX3 by altering the DNA methylation of the RUNX3 in PCa cells.

Conclusion: RUNX3 is hypermethylated in a panel of PCa cell lines; Inhibits DNA methylation of RUNX3 could restored its gene expression, which in turn induced its anti-cancer effects. Thus, RUNX3 may serve as a novel putative molecular target gene for PCa therapy.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

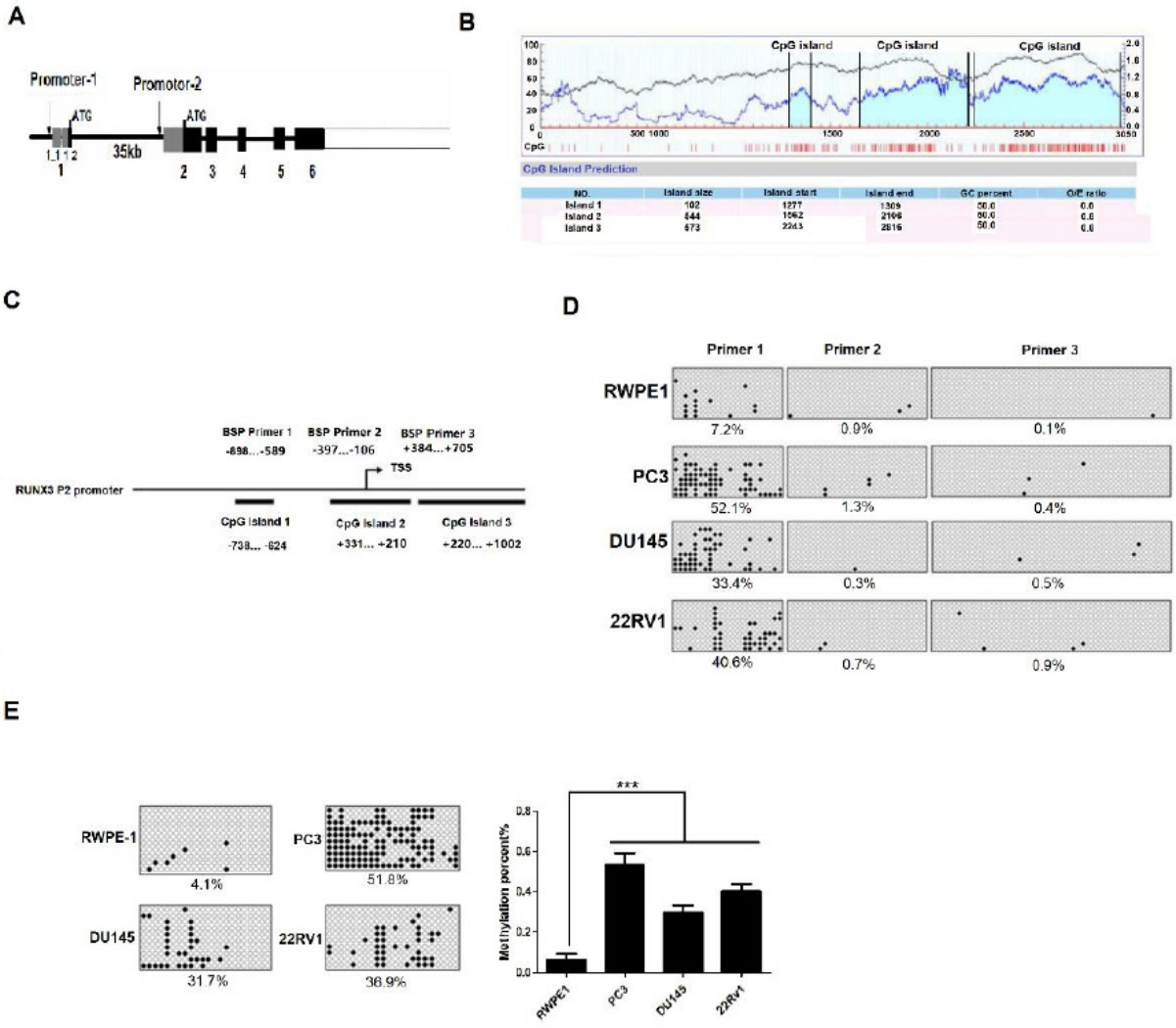

Figure 1

DNA hypermethylation of the P2 promoter region of RUNX3 in PCa cells. A: The RUNX3 structure; B: The sites of CpG island in the P2 promoter region of RUNX3; C: the schematic representation of BSP primer designing; D: 3 pairs of BSP primers were used to detect the gene methylation status of RUNX3 in RWPE1, PC3, DU145, and 22Rv1 cells; E: Primer 1 was used to detect and analyze the gene methylation levels of RUNX3 in RWPE1, PC3, DU145, and 22Rv1 cells; *** P <0.0001.

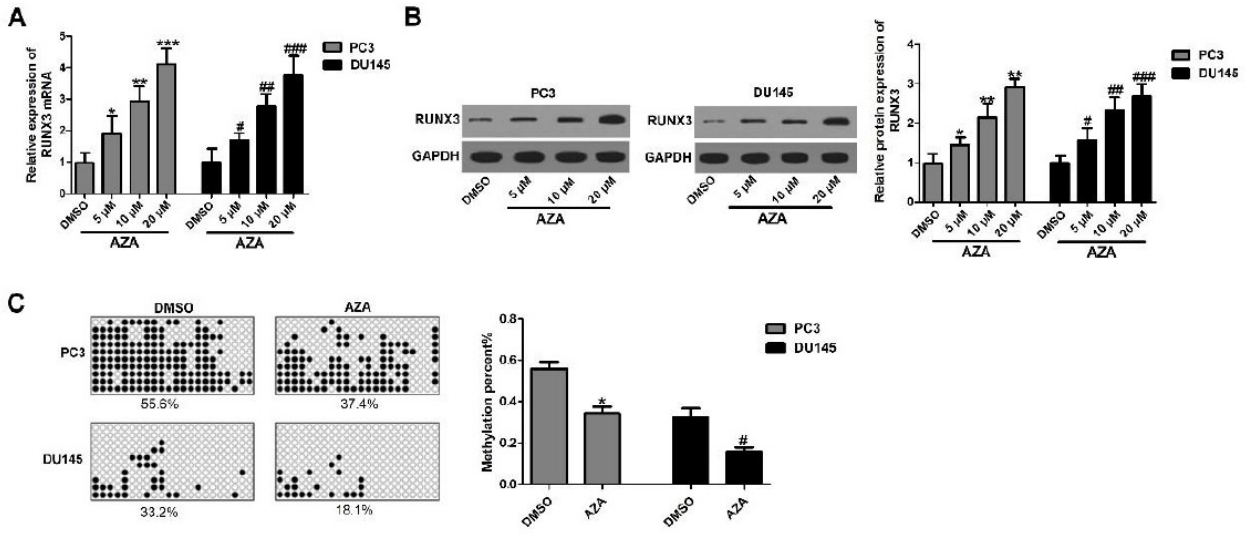

Figure 2

AZA demethylated the RUNX3 promoter region and promoted the RUNX3 expression in PCa cells. A: AZA promoted the expression of RUNX3 mRNA in PCa cells PC3 and DU145; B: AZA promoted the expression of RUNX3 protein in PCa cells PC3 and DU145; C: AZA demethylated the promoter of RUNX3 in PC3 and DU145; compared with DMSO group in PC3 cells, * P <0.05, ** P <0.01, *** P <0.001; compared with DMSO group in DU145 cells #P <0.05, ## P <0.01, ### P <0.001.

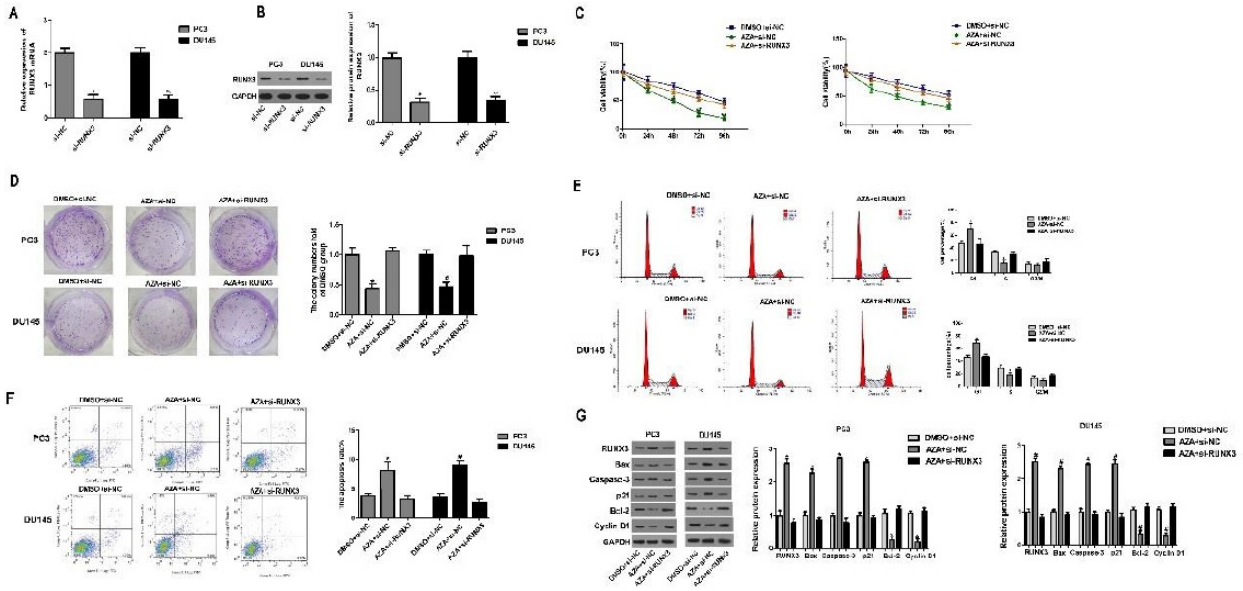

Figure 3

AZA inhibited the proliferation and induced apoptosis of PCa cells by up-regulating the expression of RUNX3 A: The effect of transfection with si-RNA targeting RUNX3 on the mRNA expression of RUNX3 in PC3 and DU145 cells as detected by qRT-PCR; B: the effect of the transfection with si-RNA targeting RUNX3 on the protein expression of RUNX3 in PC3 and DU145 cells as detected by Western blot assay; C: AZA inhibited the proliferation of PCa cell lines PC3 and DU145; D: AZA inhibited the colony-forming capability of PCa cell lines PC3 and DU145; E: AZA inhibited the cell cycle progression of PCa cell lines PC3 and DU145; F: AZA promoted the apoptosis of PCa cell lines PC3 and DU145; G: Western blot assay revealed that AZA promoted the protein expression of RUNX3, Bax, caspase-3, and p21 in PCa cell lines PC3 and DU145, and inhibited the protein expression of Bcl-2 and cyclinB1; compared with DMSO + si-NC group PC3 cells * P <0.05, ** P <0.01; compared with DMSO + si-NC group DU145 cells, #P <0.05.

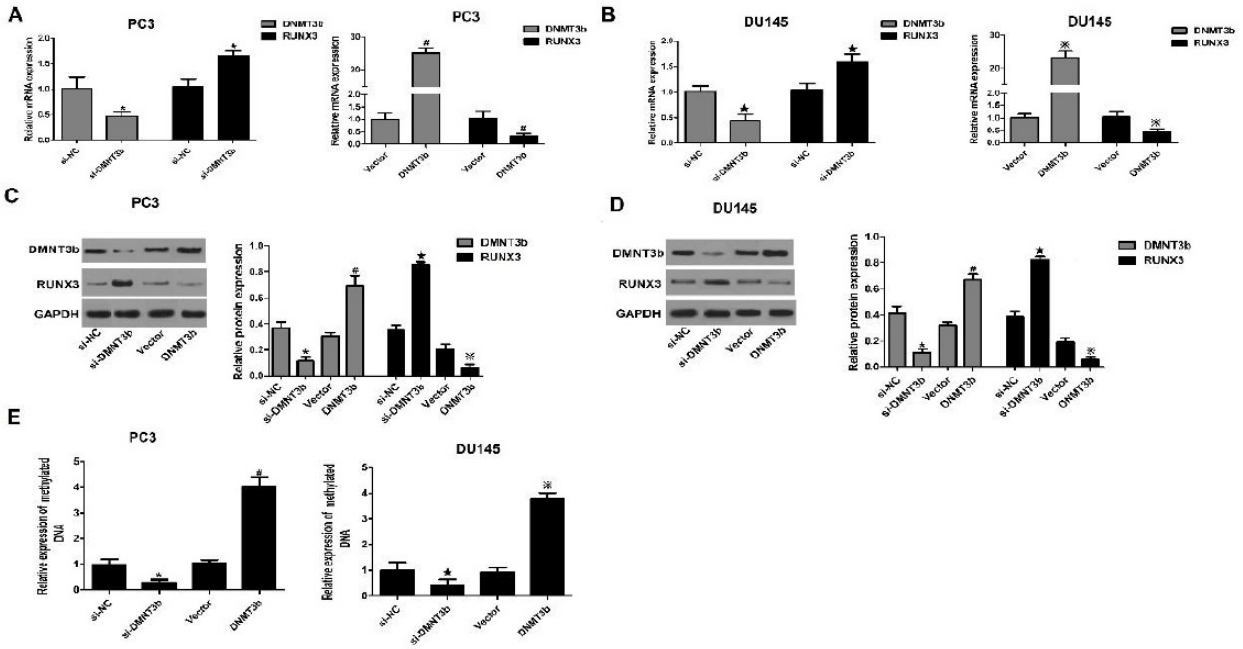

Figure 4

DNMT3b is a critical factor affecting the DNA methylation of RUNX3 in PCa cells. A, B: The effect of the knockout or overexpression of DNMT3b in PC3 and DU145 cells on the expression of DNMT3b and RUNX3 mRNAs as detected by qRT-PCR; C, D: the effect of the knockout or overexpression of DNMT3b in PC3 and DU145 cells on the protein expression of DNMT3b and RUNX3 as detected by Western blot assay; E: the effect of the knockout or overexpression of DNMT3b in PC3 and DU145 cells on the methylation level of RUNX3 in PC3 and DU145 cells as detected by MSP test; compared with si-NC group PC3 cells *P <0.05; compared with the Vector group PC3 cells, #P <0.05; compared with the si-NC group DU145 cells, ★P <0.05; compared with the Vector group DU145 cells, ※P <0.05.