D J Med Sci 2025;11(2):88-91
doi: 10.5606/fng.btd.2025.192

Original Article

The ZEB1-AS1/ZEB1 axis promotes epithelial-mesenchymal transition in prostate cancer

Gamze Nur Öter[1], Merve Nur Işık Kolukısaoğlu[2], İlknur Bingül[2], Servet Tunoğlu[3], Canan Küçükgergin[2]

[1]*Department of Medical Biochemistry, İstanbul University, Institute of Health Sciences, İstanbul, Türkiye*
[2]*Department of Medical Biochemistry, İstanbul University, Faculty of Medicine, İstanbul, Türkiye*
[3]*Department of Medical Biology and Genetic, Demiroğlu Science University, Faculty of Medicine, İstanbul, Türkiye*

ABSTRACT

Objectives: Our purpose was to evaluate the expression of long non-coding RNA (lncRNA) zinc finger E-box binding homeobox 1-antisense 1 (ZEB1-AS1) and the transcription factor ZEB1 in prostate cancer (PCa) cells, and to explore their potential role in tumor aggressiveness and epithelial-mesenchymal transition (EMT) regulation.

Materials and methods: PC3, DU145, LNCaP, and PNT1A were cultured under standard conditions. miRNeasy Tissue/Cells Advanced Mini Kit was used to isolate total RNA. RT^2 First Strand Kit was used to synthesize complementary DNA for lncRNAs, and miRCURY LNA RT Kit for microRNAs (miRNAs). Rotor-Gene Q (Qiagen) system was used to carry out quantitative real-time PCR (qRT-PCR) using SYBR Green chemistry. ZEB1-AS1 and ZEB1 levels were measured in the PCa *in vitro* model.

Results: The qRT-PCR analysis revealed that ZEB1-AS1 and ZEB1 were significantly upregulated in androgen-independent PC3 and DU145 cells compared with PNT1A ($p<0.01$), while LNCaP showed lower expression levels. Elevated ZEB1-AS1 was linked to higher ZEB1 expression, especially in PC3 cells, indicating EMT activation.

Conclusion: Our findings suggest the ZEB1-AS1/ZEB1 axis has a crucial role in PCa progression by promoting EMT and metastatic potential. This regulatory network could act as a possible marker and treatment goal, particularly in aggressive, androgen-independent PCa subtypes.

Keywords: Epithelial-mesenchymal transition, PNT1A, prostate cancer, ZEB1, ZEB1-AS1.

Among men, prostate cancer (PCa) ranks as the second most frequent malignancy and continues to be a major contributor to cancer-associated mortality globally, according to GLOBOCAN 2022 estimates.[1] Despite advances in surgery, radiotherapy, hormone therapy, and chemotherapy, the incidence of PCa continues to rise. Many patients eventually experience relapse and develop metastatic disease, which is associated with poor survival rates. These challenges highlight the urgent need to better understand the molecular mechanisms that drive PCa progression in order to identify new biomarkers and therapeutic targets.

Over the past decade, genomic research has revealed that although less than 2% of the human genome encodes proteins, a large portion is transcribed into non-coding RNAs (ncRNAs).[2] Among these, lncRNAs, which are over 200 nucleotides in length and do not encode proteins, are increasingly recognized as crucial modulators of gene regulation at transcriptional, post-transcriptional, and epigenetic levels.[3,4] A particularly important role of lncRNAs involves functioning as competing endogenous RNAs (ceRNAs), where they bind to microRNAs (miRNAs) and influence downstream gene networks.[5] In cancer biology, zinc finger E-box binding homeobox proteins ZEB1 (zinc finger E-box binding homeobox 1) and ZEB2 are well-recognized drivers of epithelial-mesenchymal transition (EMT). By suppressing epithelial markers and activating mesenchymal programs,

Received: August 26, 2025
Accepted: September 01, 2025
Published online: September 26, 2025

Correspondence: Gamze Nur Öter.
E-mail: gamze.oter@istinye.edu.tr

Cite this article as:
Öter GN, Işık Kolukısaoğlu MN, Bingül İ, Tunoğlu S, Küçükgergin C. The ZEB1-AS1/ZEB1 axis promotes epithelial-mesenchymal transition in prostate cancer. D J Med Sci 2025;11(2):88-91. doi: 10.5606/fng.btd.2025.192.

these transcription factors enhance cell motility, plasticity, and metastatic potential.[6] High ZEB1 expression has been linked to advanced disease stages and poor prognosis in several cancers, including PCa.[7] Consequently, deregulation of EMT pathways is now seen as a central mechanism promoting tumor aggressiveness and resistance to therapy.

Recent studies suggest that specific lncRNAs, especially ZEB1-AS1 (antisense 1), may regulate EMT by interacting with ZEB family transcription factors. However, the precise role of ZEB1-AS1 in PCa remains not fully understood. Exploring this regulatory relationship could provide valuable insights into the molecular basis of PCa aggressiveness and offer opportunities for developing new biomarkers and targeted treatments.

MATERIALS AND METHODS

Cell culture

All studies were conducted at İstanbul University, Department of Medical Biochemistry, Research Laboratory. PC3, DU145, and LNCaP PCa cell lines (ATCC, Manassas, VA, USA), along with the immortalized normal prostate epithelial line PNT1A, were cultured at 37°C in 5% CO_2 in RPMI-1640 (10% fetal bovine serum (FBS), 1% penicillin-streptomycin (Sigma-Aldrich, Darmstadt, Germany).

RNA isolation and cDNA synthesis

Ribonucleic acid isolation was performed with the miRNeasy Tissue/Cells Kit (Qiagen, Hilden, Germany), and concentrations were determined spectrophotometrically with a NanoDrop. Complementary DNA (cDNA) was synthesized using the RT² First Strand Kit (Qiagen, Hilden, Germany); ZEB1 and ZEB1-AS1 were amplified with gene-specific primers (Qiagen, Hilden, Germany).

Quantitative real-time PCR analysis

Reactions were performed on a Rotor-gene Q system (Qiagen, Hilden, Germany) with RT² SYBR Green ROX FAST Mastermix (Qiagen, Hilden, Germany) under standard cycling conditions: *(i)* Initial denaturation 10 min 95°C, *(ii)* denaturation 15 sec 95°C, and annealing/extension 60 sec 60°C, 40 times. Melt-curve analysis verified specificity.

Statistical analysis

The relative level of gene expression was determined using the $2^{-\Delta\Delta Ct}$ method. Each experiment was repeated three times. A one-way ANOVA and a Tukey's post hoc test were conducted using GraphPad Prism version 10.0 software (GraphPad Software Inc., La Jolla, California, USA). A p-value of less than 0.05 was considered statistically significant.

RESULTS

Differential expression of ZEB1-AS1 in prostate cancer cell lines

Quantitative real-time PCR (qRT-PCR) analysis demonstrated distinct expression patterns of lncRNA ZEB1-AS1 across PCa cell lines compared with normal prostate epithelial cells (PNT1A). ZEB1-AS1 was markedly upregulated in PC3 cells (8.6-fold, $p<0.001$) and significantly increased in DU145 cells (3.2-fold, $p<0.01$*), whereas LNCaP cells exhibited relatively lower expression levels (1.4-fold, not significant), as shown in Figure 1a. These results indicate that ZEB1-AS1 expression correlates with androgen-independent and more aggressive PCa phenotypes.

ZEB1 expression in prostate cancer cell lines

Consistent with the lncRNA findings, ZEB1 mRNA expression was strongly upregulated in PC3 cells (6.9-fold, $p<0.001$) and moderately increased in DU145 cells (2.8-fold, $p<0.01$*). In contrast, LNCaP cells showed only a slight upregulation (1.2-fold, not significant) compared with PNT1A, as shown in Figure 1b. These data suggest a positive regulatory relationship between ZEB1-AS1 and ZEB1 in aggressive PCa cell lines.

Interaction between ZEB1-AS1 and ZEB1

Pearson correlation analysis showed a strong and positive relationship between ZEB1-AS1 and ZEB1 levels across the PCa cell lines ($r=0.87$, $p<0.01$), supporting the hypothesis that ZEB1-AS1 functions as a regulatory enhancer of ZEB1 expression.

DISCUSSION

Our results show that both the transcription factor ZEB1 and the lncRNA ZEB1-AS1 are

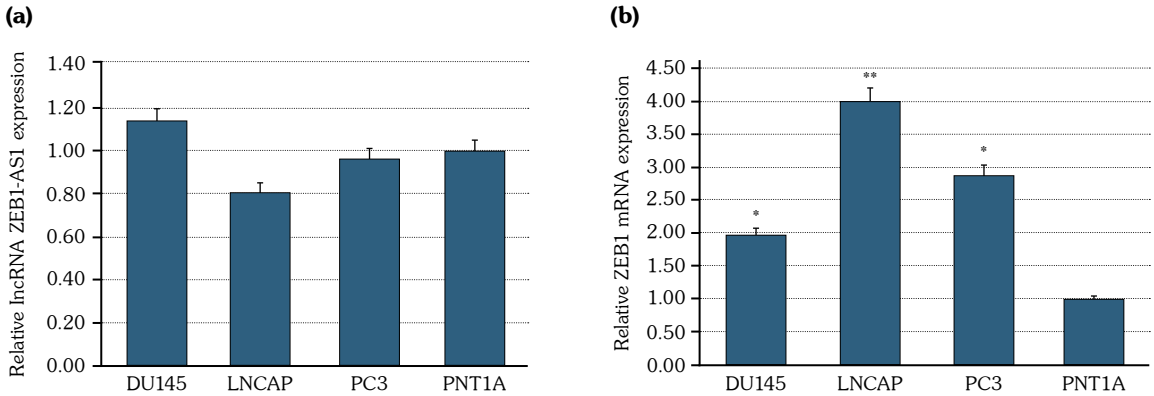

Figure 1. RT-qPCR experiment for lncRNA-ZEB1-AS1 and ZEB1. **(a)** Relative expression of ZEB1-AS1 in prostate cancer cell lines (PC3, DU145, LNCaP) compared to PNT1A. **(b)** Relative expression of ZEB1 mRNA in prostate cancer cell lines compared to PNT1A. Bars represent mean ± standard deviation of three independent experiments. Statistical significance was determined by one-way ANOVA followed by Tukey's *post-hoc* test.

* $p<0.05$; ** $p<0.01$.

significantly upregulated in aggressive, androgen-independent cells PC3 and DU145 compared with the androgen-sensitive LNCaP line and the non-malignant control PNT1A. This distinct expression pattern strongly suggests that the ZEB1-AS1/ZEB1 axis is a key driver of EMT, a critical process involved in metastasis and therapy resistance in PCa.[8,9]

On a mechanistic level, ZEB1-AS1 promotes EMT by increasing ZEB1 expression-either through epigenetic mechanisms involving the recruitment of the MLL1 (mixed lineage leukemia protein-1) complex and enrichment of H3K4me3 (tri-methylation of histone H3 at lysine 4) histone marks at the ZEB1 promoter, or by serving as a ceRNA that sequesters members of the miR-200 family.[10,11] ZEB1, in turn, facilitates EMT by repressing epithelial markers such as E-cadherin and activating genes associated with the mesenchymal phenotype, thereby enhancing cellular motility and invasion.[12] The high levels of ZEB1-AS1 and ZEB1 found in PC3 and DU145 cells correlate with their invasive behavior, while the comparatively low expression in LNCaP cells suggests that androgen receptor (AR) signaling may suppress EMT regulation. Previous research supports this, showing that AR activity can inhibit EMT-related gene expression and maintain epithelial characteristics.[13] Therefore, loss of AR signaling in castration-resistant contexts may reveal the pro-EMT effects of the ZEB1-AS1/ZEB1 axis.

Despite the strong link between ZEB1-AS1 and ZEB1 expression, the precise regulatory mechanisms are still unclear. Functional studies-such as luciferase reporter assays to test promoter activity, RNA immunoprecipitation to identify miRNA interactions, and gene knockdown experiments-are needed to determine whether ZEB1-AS1 directly regulates ZEB1 or acts through intermediary ncRNA networks.[14] From a translational perspective, the ZEB1-AS1/ZEB1 axis offers a promising biomarker and therapeutic target. In other cancers, silencing ZEB1-AS1 with small interfering RNAs or antisense oligonucleotides has been shown to suppress EMT and increase chemotherapy sensitivity.[15] This approach could be especially useful for treating castration-resistant PCa. Additionally, since circulating lncRNAs like ZEB1-AS1 are detectable in patient plasma, they could serve as non-invasive biomarkers for disease monitoring and therapy response evaluation.[16,17] However, this study has limitations. It is restricted to *in vitro* PCa models and uses only PNT1A as a normal control. To improve clinical relevance, validation should be conducted using more physiologically relevant models, such as patient-derived organoids, *in vivo* xenograft models, and large-scale transcriptomic datasets.

Moreover, measuring circulating ZEB1-AS1 levels in patient samples could help determine its diagnostic and prognostic value.

In conclusion, our data support a model where ZEB1-AS1 enhances ZEB1 expression, promotes EMT, and drives metastatic progression in PCa. The identified ZEB1-AS1/ZEB1 pathway deepens insights into PCa progression and highlights a promising direction for biomarker discovery and therapeutic intervention.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Conception/design of study, critical revision of manuscript, final approval and accountability: G.N.Ö.; Data acquisition, final approval and accountability: M.N.I.K.; Final approval and accountability, critical revision of manuscript: I.B.; Data analysis/interpretation, final approval and accountability: S.T.; Conception, critical revision of manuscript, final approval and accountability: C.K.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024;74:229-63. doi: 10.3322/caac.21834.
2. George TP, Subramanian S, Supriya MH. A brief review of noncoding RNA. Egypt J Med Hum Genet 2024;25:98. doi: 10.1186 s43042-024-00553-y.
3. Mattick JS, Amaral PP, Carninci P, Carpenter S, Chang HY, Chen LL, et al. Long non-coding RNAs: Definitions, functions, challenges and recommendations. Nat Rev Mol Cell Biol 2023;24:430-47. doi: 10.1038/s41580-022-00566-8.
4. Statello L, Guo CJ, Chen LL, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol 2021;22:96-118. doi: 10.1038/s41580-020-00315-9.
5. Sharma S, Houfani AA, Foster LJ. Pivotal functions and impact of long con-coding RNAs on cellular processes and genome integrity. J Biomed Sci 2024;31:52. doi: 10.1186/s12929-024-01038-1.
6. Huang Y, Hong W, Wei X. The molecular mechanisms and therapeutic strategies of EMT in tumor progression and metastasis. J Hematol Oncol 2022;15:129. doi: 10.1186/s13045-022-01347-8.
7. Parfenyev SE, Daks AA, Shuvalov OY, Fedorova OA, Pestov NB, Korneenko TV, et al. Dualistic role of ZEB1 and ZEB2 in tumor progression. Biol Direct 2025;20:32. doi: 10.1186/s13062-025-00604-3.
8. Goossens S, Vandamme N, Van Vlierberghe P, Berx G. EMT transcription factors in cancer development re-evaluated: Beyond EMT and MET. Biochim Biophys Acta Rev Cancer 2017;1868:584-91. doi: 10.1016/j.bbcan.2017.06.006.
9. Hsieh CL, Xie Z, Zhang H. (2022). Roles of epithelial–mesenchymal transition transcription factors in cancer drug resistance. Oncogene 2022;41:1209-24. doi: 10.1038/s41388-021-02127-0.
10. Su W, Xu M, Chen X, Chen N, Gong J, Nie L, et al. Long noncoding RNA ZEB1-AS1 epigenetically regulates the expressions of ZEB1 and downstream molecules in prostate cancer. Mol Cancer 2017;16:142. doi: 10.1186/s12943-017-0711-y.
11. Zhang X, Xu Y, Sun Y, Wang J. (2020). LncRNA ZEB1-AS1 promotes epithelial-mesenchymal transition and metastasis through miR-200 sponging in multiple cancers. J Exp Clin Cancer Res 2020;39:248. doi: 10.1186/s13046-020-01743-5.
12. Dongre A, Weinberg RA. New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. Nat Rev Mol Cell Biol 2019;20:69-84. doi: 10.1038/s41580-018-0080-4.
13. Sharp A, Coleman I, Yuan W, Sprenger C, Dolling D, Rodrigues DN, et al. Androgen receptor splice variant-7 expression emerges with castration resistance in prostate cancer. J Clin Invest 2019;129:192-208. doi: 10.1172/JCI122819.
14. Thomson DW, Dinger ME. Endogenous microRNA sponges: Evidence and controversy. Nat Rev Genet 2016;17:272-83. doi: 10.1038/nrg.2016.20.
15. Zhou W, Chen S, Li Y. Targeting lncRNA-mediated EMT with antisense oligonucleotides: A novel therapeutic strategy in oncology. Mol Ther Nucleic Acids 2024;34:102022. doi: 10.1016/j.omtn.2023.102022.
16. Li J, Tian H, Zhang X. (2024). Exosomal fg RNAs in prostate cancer: Emerging roles as biomarkers and therapeutic targets. Transl Oncol 2024;32:101720. doi: 10.1016/j.tranon.2024.101720.
17. Jalali S, Yıldız G, Demir Ö. Circulating lncRNAs as non-invasive biomarkers for prostate cancer diagnosis and prognosis. Front Oncol 2021;11:761979. doi: 10.3389/fonc.2021.761979.