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Deciphering the prognostic significance and regulatory networks of ZEB1 and ZEB2 in prostate adenocarcinoma

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ABSTRACT

Aim: Prostate adenocarcinoma (PRAD), a prevalent malignancy affecting men globally, represents a complex interplay of genetic, epigenetic, and microenvironmental influences. Uncontrolled expression of Zinc finger E-box-binding homeobox (ZEB) genes lead to uncontrolled cell division, a characteristic feature of malignancy and cause to evading immune surveillance and establishing a pro-tumorigenic microenvironment. This study aimed to comprehensively investigate the prognostic significance and regulatory roles of ZEB1 and ZEB2 in PRAD.

Methods: Bioinformatic analyses utilizing TCGA database data and validation with TCGA-PRAD patient datasets were conducted. Expression patterns of ZEB1 and ZEB2 across cancers were explored, followed by survival analyses in PRAD. The association with clinical parameters, such as Gleason score, metastasis, and TP53 mutation, was investigated using the UALCAN and GEPIA databases. Protein expression was validated through the Human Protein Atlas. A protein-protein interaction (PPI) network analysis elucidated regulatory landscapes.

Results: ZEB1 and ZEB2 showed diverse expression across cancers, with decreased expression in PRAD. Survival analyses confirmed their prognostic relevance in PRAD. Correlation with Gleason score and metastasis highlighted their clinical significance. Protein expression analyses and PPI networks revealed interconnected regulatory pathways involving ZEB1 and ZEB2.

Conclusions: This study unveils ZEB family as potential prognostic markers for PRAD, shedding light on their complex roles in cancer biology. The identified regulatory pathways offer therapeutic targets for disrupting ZEB-mediated processes, suggesting avenues for PRAD treatment. These findings contribute to understanding the intricate landscape of ZEB family in prostate cancer and other malignancies.

Key words: Prostate adenocarcinoma, Zinc finger E-box-binding homeobox 1, Zinc finger E-box-binding homeobox 2, Gleason's score, Prognostic markers, Bioinformatic analyses, Protein-protein interaction network.

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Introduction

Prostate adenocarcinoma (PRAD), a prevalent malignancy affecting men globally, represents a complex interplay of genetic, environmental, and hormonal factors [1]. PRAD typically begins as a slow-growing tumor confined to the prostate gland. However, in some cases, the disease progresses to an aggressive state, spreading to

surrounding tissues and distant organs [2]. The factors governing this transition from localized to advanced disease are complex and multifactorial, involving genetic, epigenetic, and microenvironmental influences [3-5].

The ZEB (Zinc finger E-box-binding homeobox) family, comprising primarily of *ZEB1* and *ZEB2* (also referred to as *SIP1* or Smad-interacting protein 1), represents a group of transcription factors notable for their role in advancing cancer metastasis through the initiation of Epithelial-Mesenchymal Transition (EMT), [6]. These factors are particularly influential in the advanced stages of cancer, especially concerning tumor invasion and metastasis. *ZEB1* and *ZEB2* are instrumental in degrading the extracellular matrix (ECM), which facilitates the spread of cancer cells into adjacent tissues [7-9]. Moreover, these transcription factors enhance the cell's ability to intrastate into blood or lymphatic vessels, fostering distant metastasis. The intricate network of ZEB-mediated signaling pathways contributes significantly to establishing secondary tumor foci in distant organs [10]. Dysregulation of these processes can lead to uncontrolled cell division, a characteristic feature of malignancy. Additionally, ZEB-mediated signaling pathways contribute to evading immune surveillance and establishing a pro-tumorigenic microenvironment [11].

Given the pivotal role of the ZEB family in driving various aspects of cancer progression, these proteins have garnered attention as potential therapeutic targets. Targeting *ZEB1* and *ZEB2* may disrupt the signaling cascades that promote epithelial-mesenchymal transition (EMT), inhibit cell proliferation, and impede metastatic spread [6-8]. Beyond EMT, *ZEB1* and *ZEB2* impact various cellular processes, including cell proliferation, apoptosis, and the establishment of the pre-metastatic niche, thereby playing a pivotal role in the metastatic cascade common to many cancer types. Several preclinical studies and ongoing clinical trials explore the feasibility of developing drugs targeting the ZEB family, offering a glimpse into a promising avenue for cancer treatment [12]. However, there are no studies on the role of *ZEB1* and *ZEB2*, which belong to the ZEB family, in prostate cancer and the associated genes. Here, it is demonstrated that the ZEB family exhibits a tendency to be downregulated in numerous cancers, including prostate cancer patients. Furthermore, a reduction in ZEB expression is observed compared to normal samples, as indicated by factors such as the Gleason score, metastasis status, and molecular signature of the disease. In addition, immunohistochemistry data demonstrated that elevation in protein levels holds prognostic value exclusively within cancer, providing diagnostic significance in identifying PRAD disease. As a result of the enrichment analyses conducted, protein-protein interactions were explored, revealing that ten genes interact with the ZEB family and play a significant role in disease development. This study provides a comprehensive overview of the general role of the ZEB family in PRAD before delving into its specific implications in prostate cancer. The findings illuminate molecular mechanisms, clinical implications, and potential diagnostic avenues associated with the interaction between the ZEB family and the identified genes in the context of PRAD.

Materials and metods

Differential gene and protein expression analysis*:* The Cancer Genome Atlas (TCGA) serves as a pivotal repository for extensive experimental cancer data, encompassing diverse human tumor types. This study employed the Gene Expression Profiling Interactive Analysis (GEPIA) software to delineate the gene

expression patterns of *ZEB1* and *ZEB2* in a spectrum of human malignancies and corresponding non-cancerous tissues, with a specific focus on PRAD [13]. Utilizing the GEPIA tool, which draws upon TCGA data, we conducted an in-depth analysis to investigate the correlation between the expression of *ZEB1* and *ZEB2* and the prognosis of patients with PRAD. The GEPIA tool, accessible at http://gepia.cancer-pku.cn/, facilitated this examination.

Subsequently, confirmation of the results and an exploration of the alterations in *ZEB1* and *ZEB2* expression across different states of PRAD were undertaken using the UALCAN database (https://ualcan.path.uab.edu/cgi-bin/ualcan-res.pl) [14]. This comprehensive approach aimed to rigorously assess the expression dynamics of *ZEB1* and *ZEB2* in PRAD, employing two distinct databases to enhance the reliability and robustness of our findings.

Immunohistochemistry staining of ZEB1 and ZEB2: The validation of the immunohistochemistry (IHC) profiles of ZEB1 and ZEB2, I performed The Human Protein Atlas (HPA) is an accessible platform containing detailed immunohistochemical results, encompassing microarrays from 44 normal tissues across 20 different cancer types from 216 cancer patients and 144 healthy individuals (https://www.proteinatlas.org) [15].

In the context of PRAD, an assessment of protein expression for ZEB1 and ZEB2 was conducted. Antibodies CAB079943, HPA027524, and HPA003456 were used for this investigation's antibody-based analysis. Details such as the name of antibody, staining levels (not detected, low, high or medium), intensity, quality of the IHC analysis data and tissue type were extracted from the database to interpret the results.

Identification of potential target genes related to ZEB1 and ZEB2: In the pursuit of identifying potential target genes for both *ZEB1* and *ZEB2*, three databases were utilized: IntAct [16], (https://www.ebi.ac.uk/intact/), STRING [17], (https://string-db.org/), and BioGRID [18], (https://thebiogrid.org/). To enhance prediction accuracy and facilitate the visualization of shared targets, emphasis was placed on genes identified in at least two of the three databases. Subsequently, Venn diagrams were created to visually represent the overlapping target genes between *ZEB1* and *ZEB2*, offering a comprehensive view of their shared regulatory networks.

Performing an enrichment analysis on the shared target genes within the signaling pathways of both ZEB1 and ZEB2: An enrichment analysis on the shared target genes within the *ZEB1* and *ZEB2* signaling pathways was performed using Metascape [19] (http://metascape.org/gp/index.html). The study focused on clarifying the roles of these genes in cancer-related biological processes by conducting a Gene Ontology (GO) enrichment analysis. Valuable insights into the potential roles and contributions of the common target genes in the molecular mechanisms orchestrated by *ZEB1* and *ZEB2* in various cellular processes, particularly in the context of cancer progression and other related biological events, were provided by this enrichment analysis.

Data analysis: In this study, the following standards were applied to denote statistical significance (p values): $p > 0.05$ indicates no significant difference (ns); $p \leq 0.05$ is marked with *, $p \leq 0.01$ with **, $p \leq 0.001$ with ***, and $p \leq 0.0001$ with ****.

Results

Differential expression of ZEB1 and ZEB2 in diverse cancers: First, mRNA levels of the

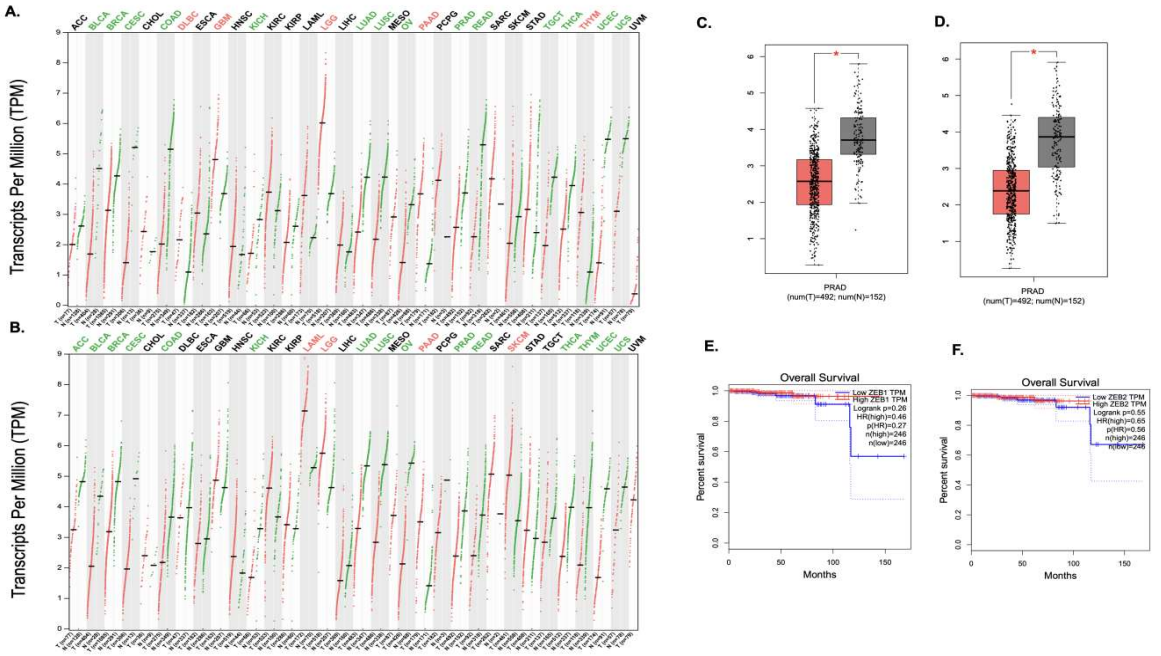

Figure 1. Differential expression of *ZEB1* and *ZEB2* in various cancers **A.** *ZEB1* expression profile across all tumor samples and paired normal tissues. **B.** *ZEB2* expression profile across all tumor samples and paired normal tissues. **C.** The expression level of *ZEB1* in PRAD tissues and adjacent non-tumor tissues. **D.** The expression level of *ZEB2* in PRAD tissues and adjacent non-tumor tissues. **E.** The overall survival of human PRAD patients concerning high or low expression levels of *ZEB1*. **F.** The overall survival of human PRAD patients with high or low expression levels of ZEB2.

ZEB family of genes (*ZEB1* and *ZEB2*) were compared between normal and tumor samples in PRAD. As shown in Fig. 1A, *ZEB1* showed an enhanced expression profile in many human cancers, such as low-grade gliomas (LGG), glioblastoma multiforme (GBM), pancreatic adenocarcinoma(PAAD), diffuse large B cell lymphoma (DLBC), Thymoma (THYM) while has lower expression in kidney chromophobe (KICH), breast invasive carcinoma (BRCA), urothelial bladder carcinoma (BLCA), colon adenocarcinoma (COAD), lung adenocarcinoma (LUAD), cervical squamous cell carcinoma (CESC), uterine carcinosarcoma (UCS), lung adenocarcinoma (LUSC), PRAD, rectum adenocarcinoma (READ), uterine corpus endometrial carcinoma (UCEC), thyroid carcinoma (THCA), testicular germ cell tumors (TGCT), and ovarian cancer (OV), (Fig. 1A). When the expression of the *ZEB2* gene in cancer and normal tissues was examined an increase was observed in acute myeloid leukemia (LAML), LGG, pancreatic adenocarcinoma (PAAD), and human skin cutaneous melanoma (SKCM) cancer patients. However, although the decreased expression profile is similar to *ZEB1*, *ZEB2* is reduced in adrenocortical carcinoma (ACC) and thymoma (THYM) patients (Fig. 1B). The GEPIA tool was used to compare the expression of the ZEB family in 492 prostate cancer patients and 152 normal individuals to confirm results for PRAD patients. As a result, it was detected that the expression of both genes decreased in PRAD patients (Fig. 2C-2D). To explore the prognostic worthiness of the ZEB family, GEPIA was also performed for survival analyses. The detailed gene expressions are given in Supplementary Table 1. As expected, as shown in Fig 1E-1F, Even though the changes were not statistically significant, the observed trend leaned towards reduced survival, with a p (HR) of 0.27 for ZEB1 and p(HR) of 0.56 for ZEB2 UALCAN database to deeply understand the effect of genes related to the TCGA-PRAD cohort based on Gleason

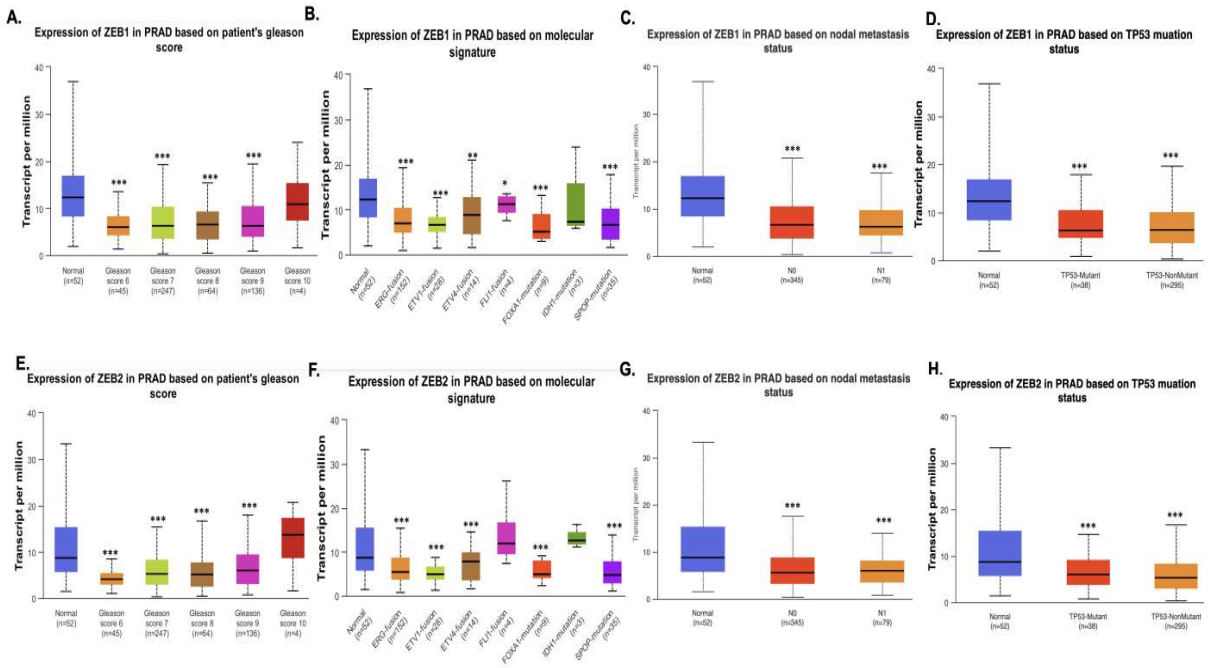

Figure 2. Differential expression of ZEB1 and ZEB2 based on different PRA**D A.** *ZEB1* expression conditions based on patients' Gleason score. **B.** *ZEB1* expression based on molecular signature. **C.***ZEB1* expression is based on nodule metastasis status. **D.** *ZEB1* expression is based on TP53 mutation. **E.** *ZEB2* expression based on patient Gleason score. **F.** *ZEB2* expression based on molecular signature. **G.** *ZEB2* expression based on nodule metastasis status. H**.** *ZEB2* expression based on TP53 mutation.

score, molecular signature, metastasis status, and P53 mutation. According to the results, the lowest expression of the *ZEB1* gene was observed in Grade IV prostate cancer patients with a Gleason score of 8. When comparing the groups based on molecular signature, *ZEB1* decreased the station *FOXP1* and *SPOP* mutation. The expression trend of the *ZEB2* gene was noted to exhibit a pattern analogous to that of *ZEB1*. The detailed gene expressions are given in Supplementary Table 2-3.

Immunohistochemical staining of ZEB 1 and ZEB 2 protein in PRAD specimens*:* Subsequently, to verify previous results, the Human Protein Atlas (HPA) database was used to analyze the protein expression of the ZEB1 and ZEB2. ZEB1 protein expression in 2 PRAD specimens, by immunohistochemical staining, indicated that the protein expression of ZEB1 was higher and more detected in PRAD patient tissues than in normal tissues (Fig 3A-C) likewise, the protein expression of ZEB2 was higher in adenocarcinoma, but medium detection in PRAD samples than normal tissues (Fig. 3D-E). These results indicated that the ZEB family might be a prognostic marker for PRAD cancer.

The prediction and screening of ZEB1 and ZEB2 genes in the regulatory networks*:* In this study, an exhaustive analysis using prominent interaction databases, including IntAct, BioGRID, and STRING, was conducted to delineate potential target genes for *ZEB1* and *ZEB2*. For ZEB1, the analysis yielded 3 genes from IntAct, 135 from BioGRID, and 12 from STRING. The subsequent selection of genes overlapping across all three databases resulted in a refined list of 32 potential target genes for *ZEB1*, as visually represented in Figure 4. To ensure the robustness of our findings, the same approach was applied to *ZEB2*, revealing 16 genes from IntAct, 61 from BioGRID, and 11 from STRING. The intersection of these datasets identified 11 predictable genes associated with *ZEB2*, as illustrated in Fig. 5A-C. A Venn

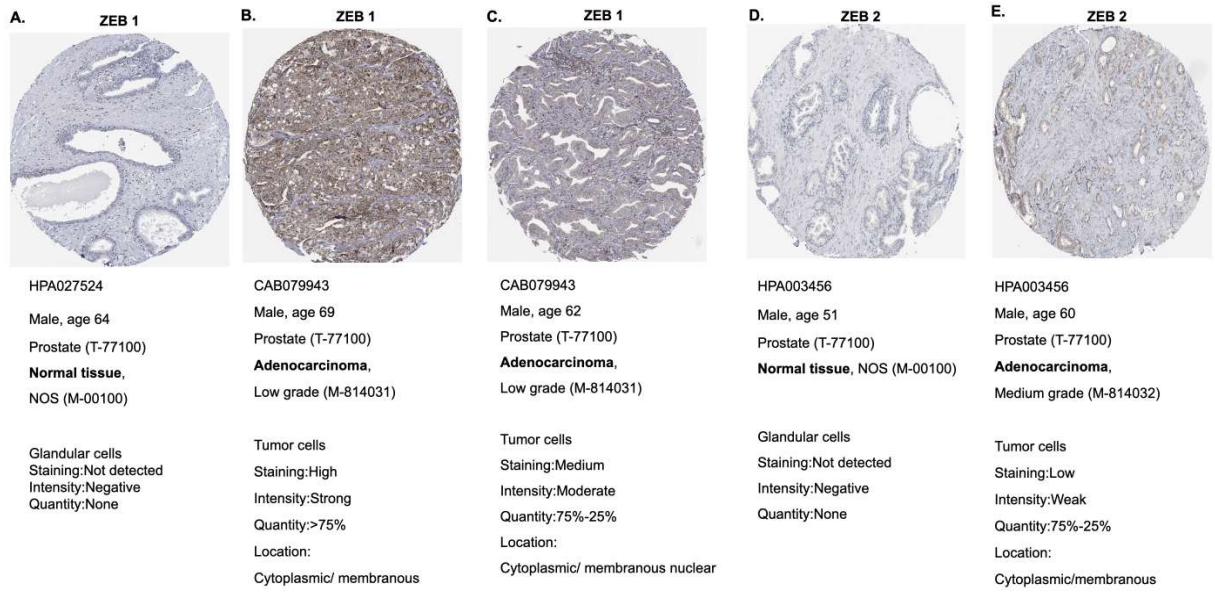

Figure 3. Immunohistochemical staining of ZEB 1 and ZEB 2 protein in PRAD specimens **A.** ZEB1 protein expression based on normal prostate tissue of the patient. **B.** ZEB1 protein expression in prostate adenocarcinoma tissue of patient **C.** ZEB1 protein expression in prostate adenocarcinoma tissue of patient **D.** ZEB2 protein expression based on normal prostate tissue patients. **E.** ZEB2 protein expression in prostate adenocarcinoma tissue of patient.

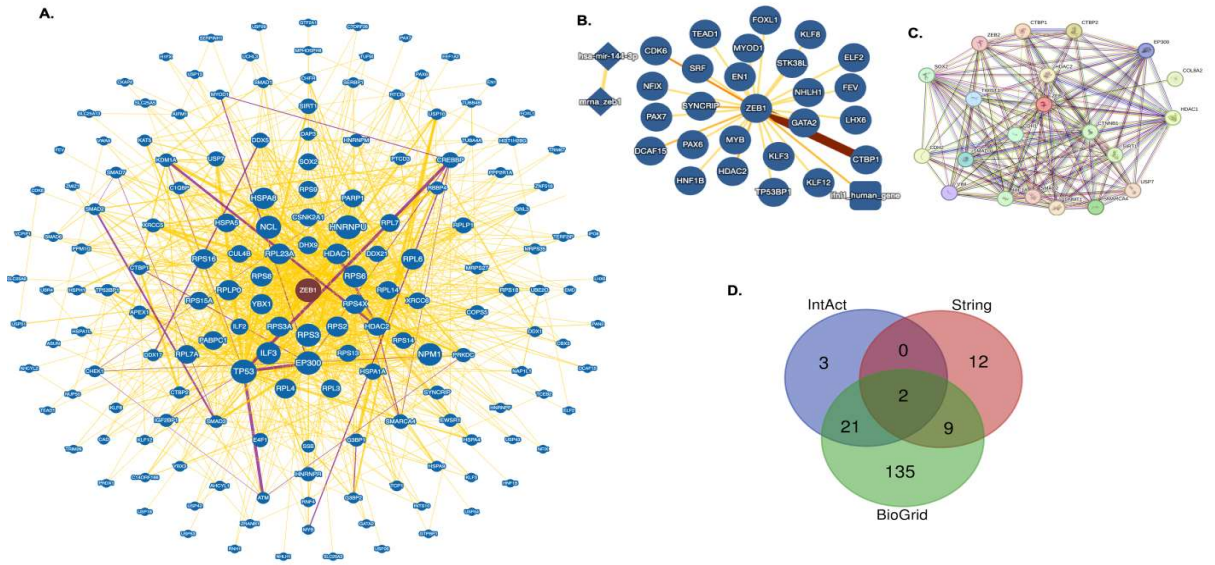

Figure 4. Prediction and screening of target genes for ZEB1 **A.** BioGRID database showing the interaction of ZEB1 with other interactions proteins. **B.** The IntAct database shows the interaction of ZEB1 with other interaction proteins. **A.** STRING database showing the interaction of ZEB1 with other interactions proteins. **D.** Venn diagram of predicted target genes from three databases.

diagram further emphasized the commonality and distinctiveness of the target genes for *ZEB1* and *ZEB2 (Fig. 5D)*. This integrative approach enhances our understanding of the potential downstream effectors of *ZEB1* and *ZEB2*, contributing to the elucidation of their roles in cellular processes and offering valuable insights for future functional studies.

Functional Diversity of ZEB1 and ZEB2 Target Genes in PRAD: GO enrichment analyses were undertaken to delineate the functional roles of the 31 target genes associated with *ZEB1* and the 11 target genes associated with *ZEB2* in tumor biological processes. The

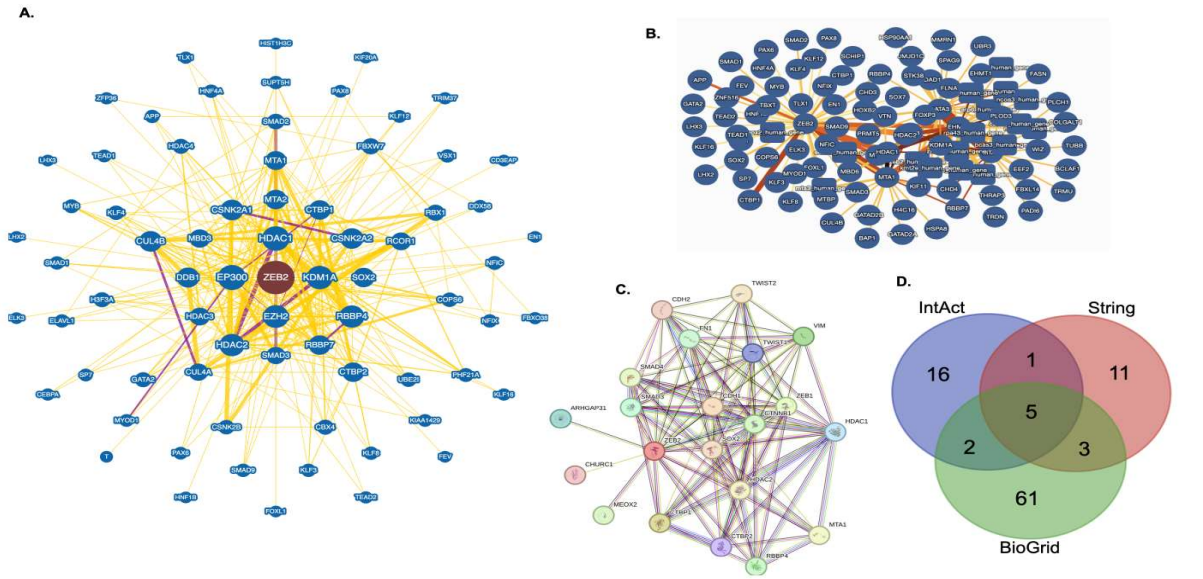

Figure 5. Prediction and screening of target genes for ZEB2 **A.** BioGRID database showing the interaction of ZEB2 with other interactions proteins. **B.** The IntAct database shows the interaction of ZEB2 with other interaction proteins. **A.** STRING database showing the interaction of ZEB2 with other interactions proteins. **D.** Venn diagram of predicted target genes from three databases.

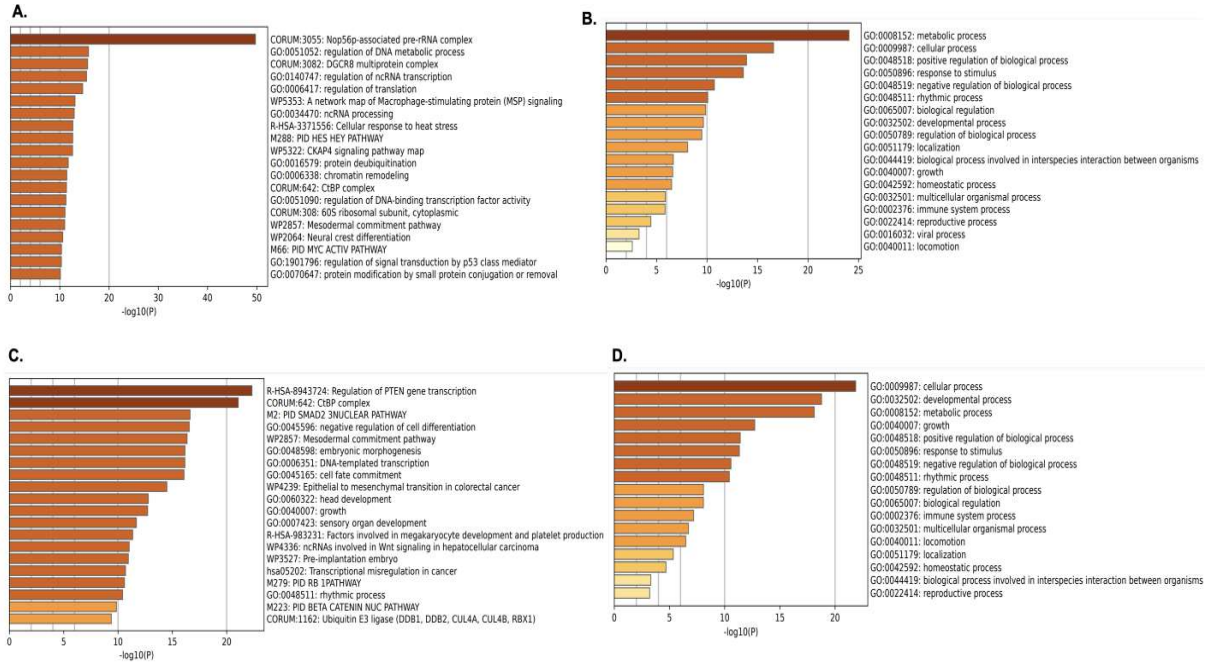

Figure 6. Gene ontology enrichment analysis of overlapping target genes for ZEB family **A.** Metascape enrichment analysis identified the most significantly enriched pathways for *ZEB1*. **B.** The top-level Gene Ontology biological processes for *ZEB1*. **C.** Metascape enrichment analysis identified the most significantly enriched pathways for ZEB2. **D.** The top-level Gene Ontology biological processes for *ZEB2.* Bar graph of strengthened terms across input gene lists, colored by *p*-values.

putative target genes of *ZEB1* manifested enrichment in several cellular *processes*, including the regulation of DNA metabolic processes, the regulation of ncRNA transcription within the *DGCR8* multiprotein complex, the regulation of translation, the modulation of Macrophage-stimulating protein (MSP) signaling in a network map, ncRNA processing,

cellular response to heat stress, the *CKAP4* signaling pathway, protein deubiquitination, chromatin remodeling, and the regulation of DNA-binding transcription factor activity (Fig. 6A).

On the other hand, the putative target genes of *ZEB2* exhibited enrichment in diverse cellular processes. These include the regulation of *PTEN* gene transcription, negative regulation of cell differentiation in *WP2857*, embryonic morphogenesis, ncRNAs involved in WNT signaling in hepatocellular carcinoma, DNA-templated transcription, epithelial to mesenchymal transition in colorectal cancer, head development, sensory organ development, factors involved in megakaryocyte development and platelet production, and transcriptional misregulation in cancer (Fig. 6B). These findings underscore the diverse and intricate roles played by *ZEB1* and *ZEB2* target genes in governing crucial cellular processes associated with tumor biology.

***Conducting a PPI network analysis to identify node degree genes among the common target genes*:** The exploration of the interaction between overlapping target genes involved the construction of a protein-protein interaction (PPI) network using Metascape. Within the PPI network, 15 genes exhibited a notable connectivity associated with *ZEB1*. These genes include E*LF2, PAX6, CTBP1, CTBP2, SOX2, SMAD2, SMARCA4, SMAD3, MYOD1, HDAC2, GATA2, HDAC1, USP7, SIRT1,* and *EP300*. Concurrently, for ZEB2, 17 genes displayed significant node degrees. These genes are *ZEB1, CTBP1, CTNND1, GATA3, VIM, TWIST1, HDAC1, CHD3, MTA2, MBD3, CTBP2, MTA3, SOX3, EHMT2, RBBP3*, *MTA3*, and *SMAD4*. All figures can be found in Supp. Fig 1. This intricate network sheds light on the interconnectedness of these genes concerning *ZEB1* and *ZEB2*, offering valuable insights into potential regulatory pathways and molecular interactions.

Discussion

ZEB1 and *ZEB2*, two genes, play a role in various signaling pathways that are associated with cancer development. These include the TGF-β, Wnt, and Notch signaling pathways. These pathways are known to regulate various aspects of cell growth, differentiation, and survival. In the context of cancer, dysregulation of these pathways can contribute to tumor progression and resistance to therapy. For a more comprehensive understanding of the prognostic significance of ZEB family in PRAD, this study employed bioinformatic analyses leveraging data from the TCGA database. The findings were subsequently validated through examination of TCGA-PRAD patient datasets, enhancing the robustness and reliability of the observed correlations.

ZEB1, a gene, shows high expression levels in mesenchymal tumors, including lung cancer, glioma, breast cancer, and pancreatic cancer [6,7]. Studies indicate a positive correlation between ZEB1 levels and tumor invasiveness. Similarly, increased expression of ZEB2, another gene, has been observed in cancers of the breast, pancreas, stomach, lung, liver, and ovary [22-25]. Research by Jacob et al. revealed that in androgen-dependent prostate cancer (PCa) cell lines, such as LNCaP cells, androgens positively regulate ZEB2 expression [26]. Graham et al. examined the expression of *ZEB1* in prostate cancer cell lines and observed differences in its expression depending on the cell line [27]. In present study, initial analysis delved into the expression trend of *ZEB1* and *ZEB2* across various cancer patients, and normal individuals. *ZEB1* exhibited enhanced expression profiles in multiple cancers such as DLBC, GBM, LGG, PAAD, and THYM. Contrastingly, *ZEB1* displayed lower expression in OV, PRAD,

LUAD, BLCA, CESC, BRCA, TGCT, COAD, LUSC, THCA, READ, UCEC, and UCS. Similarly, *ZEB2* displayed varied expression profiles across cancers, with notable increases in LAML, LGG, PAAD, and SKCM. However, *ZEB2* exhibited decreased expression in PRAD, ACC, and THYM. The confirmation of decreased *ZEB1* and *ZEB2* expression in PRAD patients using the GEPIA tool highlights their potential significance in prostate cancer. In the context of prostate cancer, specifically, the downregulation of *ZEB1* and *ZEB2* could lead to reduced tumor aggressiveness, lower chances of metastasis, and potentially better responses to therapy. However, it's important to note that the effect of manipulating these transcription factors can be complex and context-dependent, varying with the type of cancer, its stage, and the tumor microenvironment. Furthermore, targeted therapies aimed at *ZEB1* and *ZEB2* are still under investigation and not yet part of standard clinical practice. The potential therapeutic benefits of targeting these factors in prostate cancer and other malignancies continue to be an area of active research. Survival analyses further underscored the prognostic relevance of ZEB family expression, with lower levels correlating with poorer survival outcomes in PRAD patients. These findings emphasize the clinical relevance of *ZEB1* and *ZEB2* in the context of prostate cancer progression.

Several risk stratification systems have been devised for PRAD, combining the most advanced clinical and pathological parameters available, including the PSA levels, clinical and pathological staging or Gleason score [28]. Despite these efforts, the existing tools still need to improve to predict outcomes accurately. Further analyses using the UALCAN database provided insights into predicting outcomes for PRAD. This study delved into the correlation between variations in the expression of ZEB genes concerning Gleason score, metastasis, and TP53 mutation. Given the established clinical significance of the Gleason score as a measure of aggressiveness, a direct association between Gleason grade and ZEB expression was anticipated. Consistent with prior research findings [27, 29, 30], our current analysis affirms a positive relationship between elevated ZEB1 expression and advanced Gleason-grade prostate adenocarcinoma. Notably, the Gleason grades exhibit discernible segregation, with Gleason 9 emerging as the most distinct phenotype based on ZEB gene expression patterns.

Additionally, the expression of specific ZEB genes changes with a higher Gleason score. A transition was observed between the metastatic state of the patient, gene expression, and the association of ZEB genes with the Gleason score. It is hypothesized that the mechanism of the decrease in ZEB profile in prostate cancer progression may have some distinct differences between normal and cancer patients. Additionally, the correlation with molecular signatures, such as *FOXP1* and *SPOP* mutation status, further highlights the diverse regulatory roles of *ZEB1* in distinct molecular subtypes of PRAD. The protein expression analysis from the HPA confirmed the overexpression of *ZEB1* and *ZEB2* proteins in PRAD specimens compared to normal tissues. The discrepancy between low expression of ZEB1 and ZEB2 in PRAD samples, as indicated by gene expression analyses, and their high expression as revealed by IHC. It has been reported that microRNAs such as miR-203, miR-200a, miR-525-5p, miR-127, miR-101-3p and miR-186-5p play a role in the EMT pathway by downregulated *ZEB1* and *ZEB2* in various cancers including gastric, breast, and colorectal [31, 32]. Despite this, the intricacies of post-transcriptional regulation, involving factors like microRNAs and RNA stability, have the potential to influence protein

synthesis. Consequently, this regulatory mechanism can lead to lower mRNA levels, the concentrations of the corresponding proteins may remain elevated, either due to increased translation efficiency or a decrease in protein degradation.

The construction and analysis of the protein-protein interaction (PPI) network involving overlapping target genes have unveiled a complex and interconnected regulatory landscape associated with *ZEB1* and *ZEB2*. For *ZEB1*, 15 genes displayed notable connectivity within the PPI network. These genes include *ELF2*, *PAX6*, *CTBP1*, *CTBP2*, *SOX2, SMAD2*, *SMARCA4*, *SMAD3*, *MYOD1*, *HDAC2*, *GATA2*, *HDAC1*, *USP7*, *SIRT1*, and *EP300*. Simultaneously, *ZEB2* exhibited connectivity with 17 genes, namely *ZEB1, CTBP1, CTNND1, GATA3, VIM*, *TWIST1, HDAC1, CHD3, MTA2, MBD3, CTBP2, MTA3, SOX3, EHMT2, RBBP3, MTA3*, and *SMAD4*. In the biological progression, ELF2, PAX6, and GATA2 are implicated in transcriptional regulation [33]. Their connectivity with *ZEB1* suggests potential roles in modulating *ZEB1*-mediated effects on gene expression. *CTBP1* and *CTBP2* genes may contribute to *ZEB1*'s transcriptional regulatory functions, influencing downstream targets in cancer progression. *SMAD2*, *SMAD3*, and *SMAD4* proteins suggest a connection to the TGF-β signaling pathway, which intersects with *ZEB1* and *ZEB2* in the regulation of EMT [34]. Histone deacetylases (HDACs) play a role in chromatin remodeling and *HDAC1* and *HDAC2* association with *ZEB1* implies potential epigenetic regulation, impacting gene expression patterns linked to cancer development [35, 36]. *MTA2*, *MTA3*, and *CHD3* are components of chromatin remodeling complexes, suggesting a role in modifying chromatin structure and potentially influencing *ZEB1* and *ZEB2* activities [37]. The identified interconnected genes within the PPI network offer a glimpse into the potential regulatory pathways influencing *ZEB1* and *ZEB2* in the context of PRAD. Understanding these interactions may provide therapeutic targets for intervention in ZEB-mediated processes, offering new avenues for PRAD treatment. The intricate network, generated using Metascape, reveals significant connectivity among various genes, shedding light on potential regulatory pathways and molecular interactions mediated by *ZEB1* and *ZEB2*.

Conclusions

In conclusion, this comprehensive analysis elucidates the multifaceted roles of *ZEB1* and *ZEB2* in cancer, with a specific emphasis on prostate cancer. The context-dependent expression patterns, prognostic significance, and regulatory interactions with target genes underscore the intricate nature of *ZEB1* and *ZEB2* in cancer biology. The intricate network of interconnected genes associated with *ZEB1* and *ZEB2* provides a foundation for further investigations into the regulatory mechanisms and molecular interactions that underlie their roles in cancer, particularly in the complex landscape of prostate adenocarcinoma. The identified genes may be potential candidates for targeted therapies to disrupt specific pathways implicated in ZEB-mediated cancer progression. The findings provide a foundation for further experimental validation and potential therapeutic interventions targeting the ZEB family in the context of prostate cancer and other malignancies.

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Ethical statement: *Since this study was an analysis evaluation, it did not require an ethics committee decision.*

Data accessibility statement*: The data used and analyzed during this study are available from the corresponding author upon reasonable request.*

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