Iran J Public Health, Vol. 52, No. 11, Nov 2023, pp.2260-2271

Review Article

Diagnostic and Prognostic Value of miR-93 in Prostate Cancer: A Meta-Analysis and Bioinformatics Analysis

***Hassane Gazzaz** [1,2], **Maha El Habchi** [3], **Mohammed El Feniche** [4], **Yassine El Aatik** [3], **Abdelghani El Ouardi** [3], **Ahmed Ameur** [5], **Abdellah Dami** [1,6]

1. *Clinical, Metabolic and Molecular Biochemistry Team, Faculty of Medicine and Pharmacy, Mohammed V University,10100 Rabat, Morocco*
2. *Higher Institute of Nursing Professions and Health Techniques of Marrakech, annex of Safi, Morocco*
3. *Research Laboratory of Psychiatry, Medical Psychology and History of Medicine, Faculty of Medicine and Pharmacy, Mohammed V University, 10100 Rabat, Morocco*
4. *Laboratory of Biostatistics, Clinical Research and Epidemiology, Faculty of Medicine and Pharmacy, Mohammed V University, 10100 Rabat, Morocco*
5. *Department of Urology, Military Hospital Mohammed V, 10045 Rabat, Morocco*
6. *Department of Biochemistry and Toxicology, Military Hospital Mohammed V, 10045 Rabat, Morocco*

***Corresponding Author:** Email: hassane_gazzaz@um5.ac.ma

(Received 15 Mar 2023; accepted 19 Jun 2023)

Abstract

Background: Accurate and non-invasive diagnostic and prognostic markers are necessary to improve patient outcomes. MicroRNAs have been proposed as relatively non-invasive and pertinent biomarkers. miR-93 has been studied for its potential as a diagnostic and prognostic marker in prostate cancer (PCa), but findings from individual studies are inconsistent. We conducted a meta-analysis of its overall differential expression in 13 PCa studies and a bioinformatics analysis to provide a comprehensive appraisal of its diagnostic and prognostic role.

Methods: We searched all published papers on miR-93 expression in PCa up to Nov 30, 2022 using PubMed, Science Direct, Web of Science, Cochrane Central Register of Controlled Trials databases. We used RevMan software to Meta-analyze the included literature. A bioinformatics analysis of genes and pathways that might be target to the effect of the mature miR-93-5p was carried out.

Results: The pooled standardized mean difference (SMD) of miR-93 expression in PCa, its area under the curve (AUC) and hazard ratio (HR) were 1.26, 95% CI [-0.34–2.86], 0.84, 95% CI [0.76 –0.93] and 1.67, 95% CI [0.98, 2.84] respectively. Bioinformatics analysis revealed that mature miR-93-5p may regulate genes such as SMAD1, SMAD7 and MAPK and the PI3K-Akt signaling pathways.

Conclusion: miR-93 has significant diagnostic and prognostic value in PCa. These findings highlight the potential of miR-93 as a non-invasive biomarker for PCa and may contribute to earlier detection and prognostic assessment. The target genes and signaling pathways regulated by miR-93 may provide insights into the underlying molecular mechanisms of PCa.

Keywords: miR-93; Prostate; Expression; Meta-analysis; Bioinformatics

Introduction

Worldwide, Prostate cancer (PCa) is a prevalent disease affecting millions of men, with high incidence rates (1). Despite advances in the precoce detection and treatment, the malignancy remains a significant cause of mortality, and then a significant challenge for the healthcare system particularly in advanced stages (2). Because of prostate-specific antigen (PSA) lacks specificity, it does not detect the PCa or necessarily predict its biochemical cancer recurrence with sufficient accuracy (3), then, accurate and non-invasive diagnostic markers are of great importance in improving patient outcomes by enabling earlier detection and prognostic assessment.

MicroRNAs (miRNAs) are non-coding RNA molecules, with short-length of approximately 21 to 23 nucleotides (4). They modulate the expression of numerous genes through the inhibition of the translation by binding particularly to the 3' untranslated region (3'-UTR) or destabilization the mRNAs (5,6). Deregulated miRNAs expression have been associated with multiple tumors, reflecting their critical biological roles (7,8). Taken into account the expression and stability of miRNAs in different fluids, they have been proposed as relatively noninvasive and pertinent biomarkers (9,10).

miR-93 has been widely studied for its differential expression in PCa and its potential as a diagnostic and prognostic marker (11–17). However, the findings from individual studies are inconsistent and the overall utility of miR-93 as a diagnostic and prognostic biomarker for PCa remains unclear.

To provide a comprehensive assessment of the diagnostic and prognostic value of miR-93 in the PCa, we conducted a meta-analysis of its pooled differential expression, area under curve (AUC) and hazard ratio in 13 PCa studies. Our study aimed to establish the magnitude of the differential expression of miR-93 in PCa relative to normal prostate tissue and subsequently determine its overall diagnostic accuracy as a marker of PCa as well as assessing its prognostic value by evaluating its ability to predict disease outcome. Additionally, a bioinformatics analysis of the genes and pathways that might subject to the effect of the mature miR-93-5p was also being run.

Materials and Methods

The study was conducted following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)(18).

We searched all published papers written in either English or French languages on miR-93 expression in PCa up to Nov 30, 2022. Using PubMed, Science Direct, Web of Science, Cochrane Central Register of Controlled Trials, and we used the combination of keywords including 'prostate neoplasm', 'prostate tumor', 'prostate cancer', 'prostate adenocarcinoma' and 'miRNA-93', 'miR-93', 'hsa-mir-93*', 'microRNA-93', 'expression', 'diagnosis', 'SROC', 'AUC' and 'prognosis'.

Selection criteria

The criteria for papers included in the analysis are: 1) Related to miR-93 expression and PCa or survival analysis, 2) Cases are confirmed by pathology, 3) English and French publications, 4) Studies concern humans only, 5) The Availability of the mean and its standard deviation for miR-93 expression in PCa cases and controls and the hazard ratio (HR) and its 95% CIs or sufficient row data to calculate them. If not, we first contacted the author and attempted to obtain them. In case author did not return. Data were extracted independently by two authors from the published graphical representations throughout the online WebPlotDigitizer tool (https://automeris.io/WebPlotDigitizer/) (19), each extraction was done twice for more precise results.

The search criteria excluded certain types of articles, when: 1) cell or animal are the subjects of the study, 2) review, experimental studies, conference abstracts, expert opinion, case report or

incomplete data, 3) articles that were only available as abstracts and lacked full text.

Data extraction and quality assessment

The data from eligible articles were extracted by two authors independently, and any discrepancy was resolved by consensus. The first author's name, publication year, country, sample type and size, mean and standard deviation, area under the ROC (receiver operating characteristics) curve (AUC), hazard ratio and 95% confidence intervals, follow-up time of the article included in this analysis were collected.

The standard Newcastle-Ottawa Scale (NOS) (20) was assessed independently by two authors who resolved any disputes through discussion and consensus in order to evaluate the quality of the studies that were included. The assessment was based on three domains: a) selection of study groups (4 items), b) comparability of study groups (2 items) and c) exposure or outcome measurement (3 items).

Statistical analysis

We used Review Manager (RevMan) 5.4.1 software to Meta-analyze the included literature. When I^2 was greater than 50% or $P<0.05$, there was a significant heterogeneity and the random-effects model was used. Otherwise, the fixed-effects model was applied. Sensitivity analysis was used to explore the source of heterogeneity by removing one study each time to verify the stability of the result. We used standardized mean difference (SMD) and 95% confidence intervals to evaluate the differential expression of miR-93 in PCa considering that there are some differences in the measure instruments and samples between the studies, the area under the curve (AUC) and its 95% CI to assess the ability of miR-93 to differentiate between prostatic disease and normal tissues. The values of individual AUC were converted by the RevMan calculator to log [AUC] and its correspondent standard error (SE) before being pooled. The hazard ratio and its 95% CI to evaluate the miR-93 prognosis value. The funnel plot was visualized to determine the presence of publication bias.

Bioinformatics analysis

Three databases, miRDB (21), Targetscan 7.2 (22) and miRPathDB 2.0 (23) were used for the prediction of target genes. Genes present in these 3 databases were considered as predicted target genes of miR-93-5p. The online tool, Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) were used for intersection analysis). Subsequently, gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and protein-protein interaction (PPI) network analysis were conducted based on the overlapping target genes of miR-93-5p to explore the possible molecular mechanisms in PCa. GO enrichment (24) that includes 3 categories: biological process, cellular component and molecular function and KEGG pathway (25) analyses were carried out via ShinyGO v0.741: Gene Ontology (http://bioinformatics.sdstate.edu/go74/). Additionally, the protein-protein interaction (PPI) network was analyzed by the search tool for the retrieval of interacting genes database (https://string-db.org/). The highest confidence of minimum required interaction score was of>0.9 for a PPI network. Disconnected nodes were hided in the network. Line thickness indicates the strength of data support. Furthermore, we used the plug-in of molecular complex detection (MCODE) app in Cytoscape 3.7.0 software (26) to extract hub genes from the PPI network. The advanced options set as degree cutoff=2, K-Core=2, and Node Score Cutoff=0.2. The expression levels and survival analysis of miR-93-5p target genes in PCa and non-tumor tissues were determined with UALCOULD (27) (http://ualcan.path.uab.edu/index.html). Finally, the LinkedOmics (28) (http://www.linkedomics.org/) Spearman's analysis tool was applied to determine the correlation between the expression levels of miR-93-5p and the potential target genes involved in notable signaling pathways.

Results

Study Characteristics

The initial search resulted in 127 articles. After screening titles and abstracts, 29 articles were thoroughly reviewed and seven were considered for meta-analysis. These articles were selected after excluding irrelevant, duplicate, overlapping, or incomplete data. The articles consisted of 13 studies: nine comparing miR-93 expression levels between PCa and normal samples, four for the calculation of the area under curve of the miR-93 and three were evaluating the prognosis value of miR-93. Figure 1 shows the retrieval process and Tables 1 and 2 list the study characteristics.

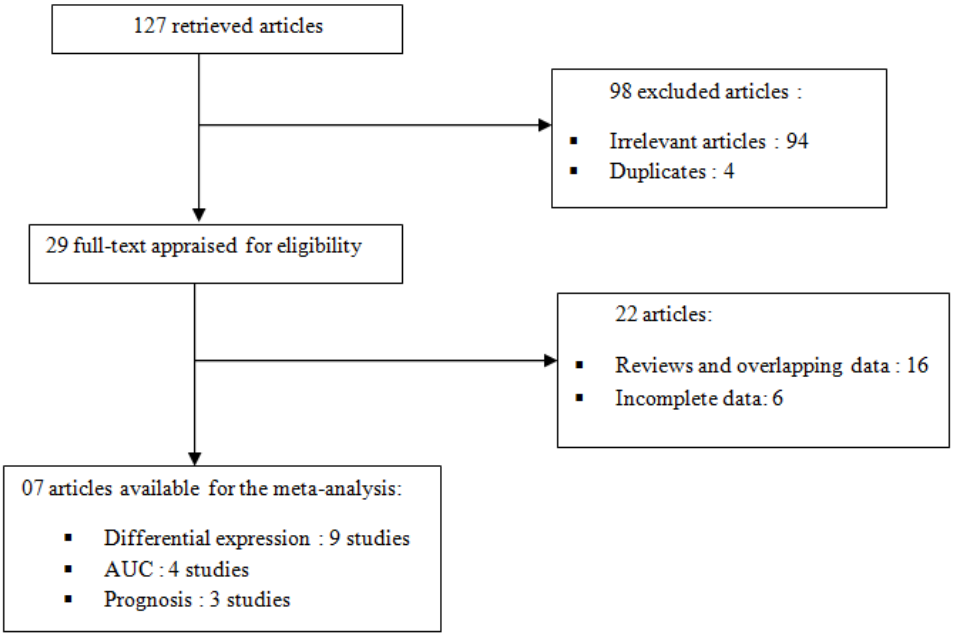

Fig.1: A PRISMA flow diagram of study eligibility and ineligibility process

Table 1: Characteristics of studies included in the diagnosis meta-analysis

Author, yr (Ref. No.)	*Country*	*Sample type*	*Case/Control*	*Test method*	*Mean case±SD*	*Mean control±SD*	*AUC [95% CI]*
Wang, 2020 (29)	China	Tissue	103/103	RT-PCR	3.76±1.76	0.97±0.56	-
Zhang1, 2021 (17)	China	Plasma	28/28	RT-PCR	-1.93±1.36	-0.86±0.76	0.78 [0.71-0.85]
Zhang2, 2021 (17)	China	Plasma	32/32	RT-PCR	-1.92±1.11	-0.74±1.02	
Porras-Quesada1, 2022 (30)	Spain	Tissue	44/28	RT-PCR	7.98±2.42	10.25±1.12	-
Porras-Quesada2, 2022 (30)	Spain	Tissue	39/28	RT-PCR	5.04±2,12	10.25± 1.12	-
Liu, 2018 (13)	China	Tissue	16/16	RT-PCR	3.00±0.43	1±0.14	-
Martínez-González1, 2021 (16)	Spain	Tissue	110/28	RT-PCR	0.012±0.003	0.001±0.0003	-
Martínez-González2, 2021 (16)	Spain	Tissue	21/28	RT-PCR	0.073±0.027	0.001±0.0003	-
Barceló, 2020 (31)	Spain	Semen	16/9	RT-PCR	7.58±1.38	5.25±1.22	0.74 [0.59-0.88]
Ciszkowicz1, 2020 (12)	Poland	Serum	40/62	RT-PCR	-	-	0.92 [0.87-0.93]
Ciszkowicz2, 2020 (12)	Poland	Tissue	26/28	RT-PCR	-	-	0.86 [0.76-0.95]

SD: standard deviation
AUC: area under curve
RT-PCR: reverse transcriptase polymerase chain reaction

Table 2: Characteristics of studies included in the prognosis meta-analysis

Reference No.	*Country*	*Sample type*	*Methods*	*Cases high/low*	*Cut-off*	*Survival*	*HR (95% CI)*	*Follow-up*
(15)	Denmark	Plassma	RT-PCR	44*	Median	OS	1.66 [0.62–4.66]	35 months
(15)	Denmark	Plasma	RT-PCR	40*	Median	OS	0.41 [0.08–1.96]	64 months
(29)	China	Tissue	RT-PCR	53/50	Median	OS	2.18 [1.09-6.82]	5 yr

*High and low are not provided separately

Literature Quality Assessment

The Newcastle–Ottawa scale used for the qualitative assessment of the included studies revealed that the total score varied from 6 to 8, with a mean of 7 for case-control studies and of 6.3 for the cohort studies. Overall, the quality of the included study was good and all 13 studies were included in the ultimate analysis (Supplementary Table 1- not published. Readers may contact the corresponding author if needed).

Meta-Analysis

The differential expression of miR-93

Based on the expression level of miR-93, nine studies from six article published from 2018 to 2022 with 709 patients were included in the meta-analysis (12,16,17,29-31). This latter revealed a standardized mean difference (SMD) of 1.26, 95% CI [-0.34–2.86, random-effects model). This suggests that miR-93 is significantly upregulated in PCa compared to normal prostate tissue. The overall effect size was found to be large and statistically significant. These results support the potential role of miR-93 as a diagnostic biomarker in PCa (Fig. 2).

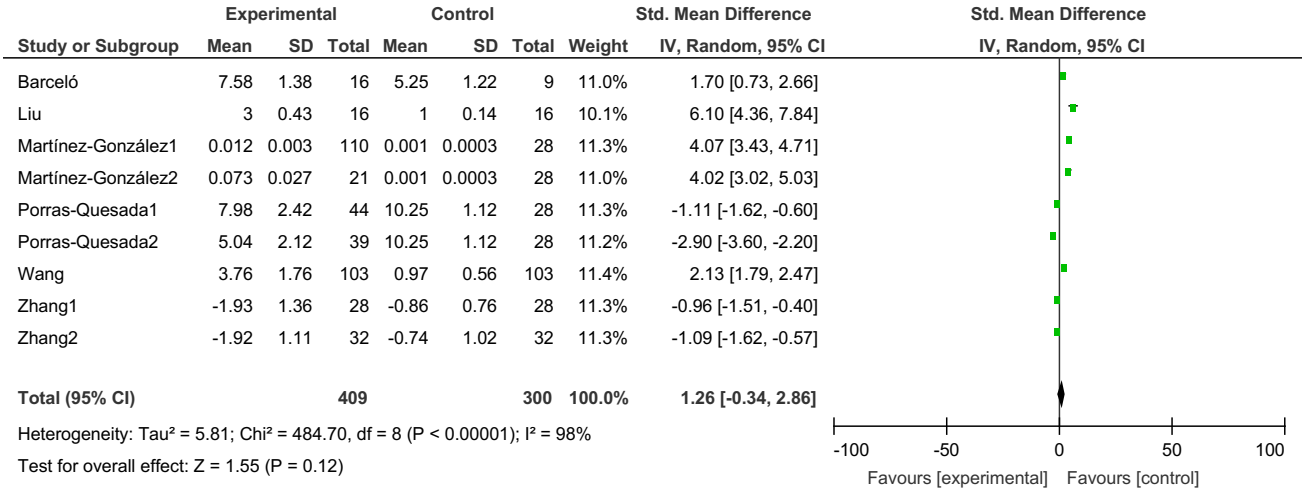

Fig. 2: Random-effects SMD for the association of miR-93 expression level and PCa

The area under the curve (AUC) of miR-93 was analyzed using data from four studies. The pooled analysis revealed an overall AUC of 0.84, 95% CI [0.76 –0.93, Random-effects model). miR-93 has a high ability to accurately differentiate between diseased and healthy tissues based on its expression levels. These findings suggest that miR-93 may have potential as a biomarker for disease diagnosis (Supplementary Fig. 1).

Prognosis value of miR-93

The meta-analysis of the prognosis miR-93 value in PCa was performed with three studies from two articles evaluating the overall-survival (OS) (15,29).

The fixed effect model was utilized to calculate the HR and 95% CI (heterogeneity: p=0.18, I^2=41%). High expression of miR-93 was associated with worse OS for PCa patients, the combined HR was 1.67, 95% CI [0.98, 2.84] (Fig. 3).

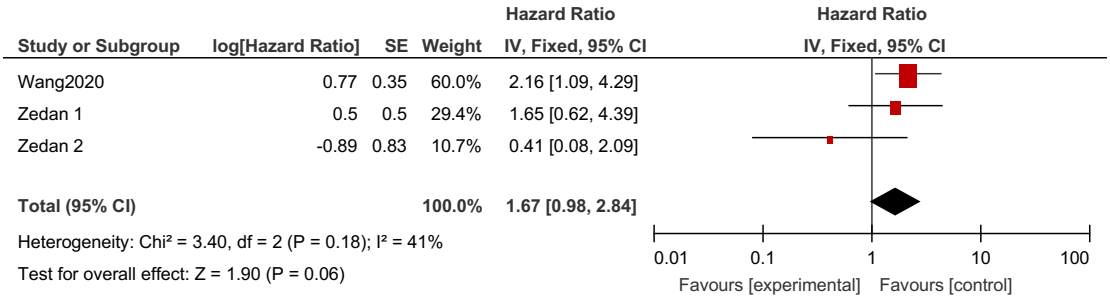

Fig. 3: Forest plot illustrating relationship between miR-93 levels and overall survival (OS) in PCa

Sensitivity and publication bias

The consistency of results was evaluated by performing a sensitivity analysis through the stepwise exclusion of individual studies, this analysis indicated that the results of the meta-analysis were stable.

Furthermore, the funnel plots were visually symmetrical, there was no obvious publication bias in any of the conducted meta-analyses. (Fig. 4A,B; Supplementary Fig. 2).

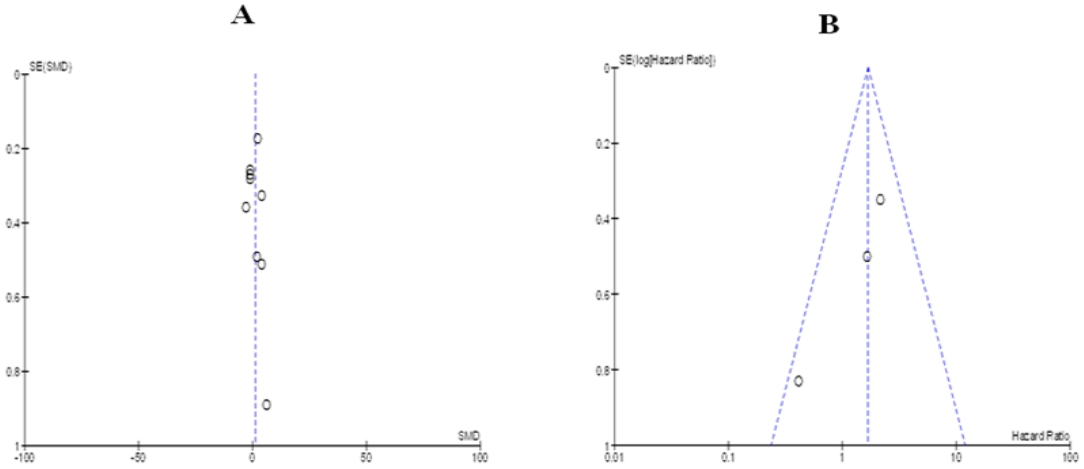

Fig. 4: Funnel plot of A) standardized mean difference, B) hazard ratio

Bioinformatics analysis

Overall, 1319 genes were predicted from miRDB, 9311 genes were predicted from miRPathDB, 1382 genes were predicted from Targetscan, and finally 879 overlapping target genes were obtained (Supplementary Fig. 3A). In bioinformatics analysis based on the overlapping target genes, the most significant and important enriched pathways from GO analysis were:

Nervous system development (GO:0007399), regulation of transcription of RNA polymerase II (GO:0006357) and transcription of RNA poly-

merase II (GO:0006366) were the top three pathways in the biological process (Fig. 5A). With respect to cellular component, the top three terms gathered by these target genes were neuronal SMAD protein complex (GO:0071141), transcription complex (GO:0005667), and chromatin (GO:0000785) (Fig. 5B).

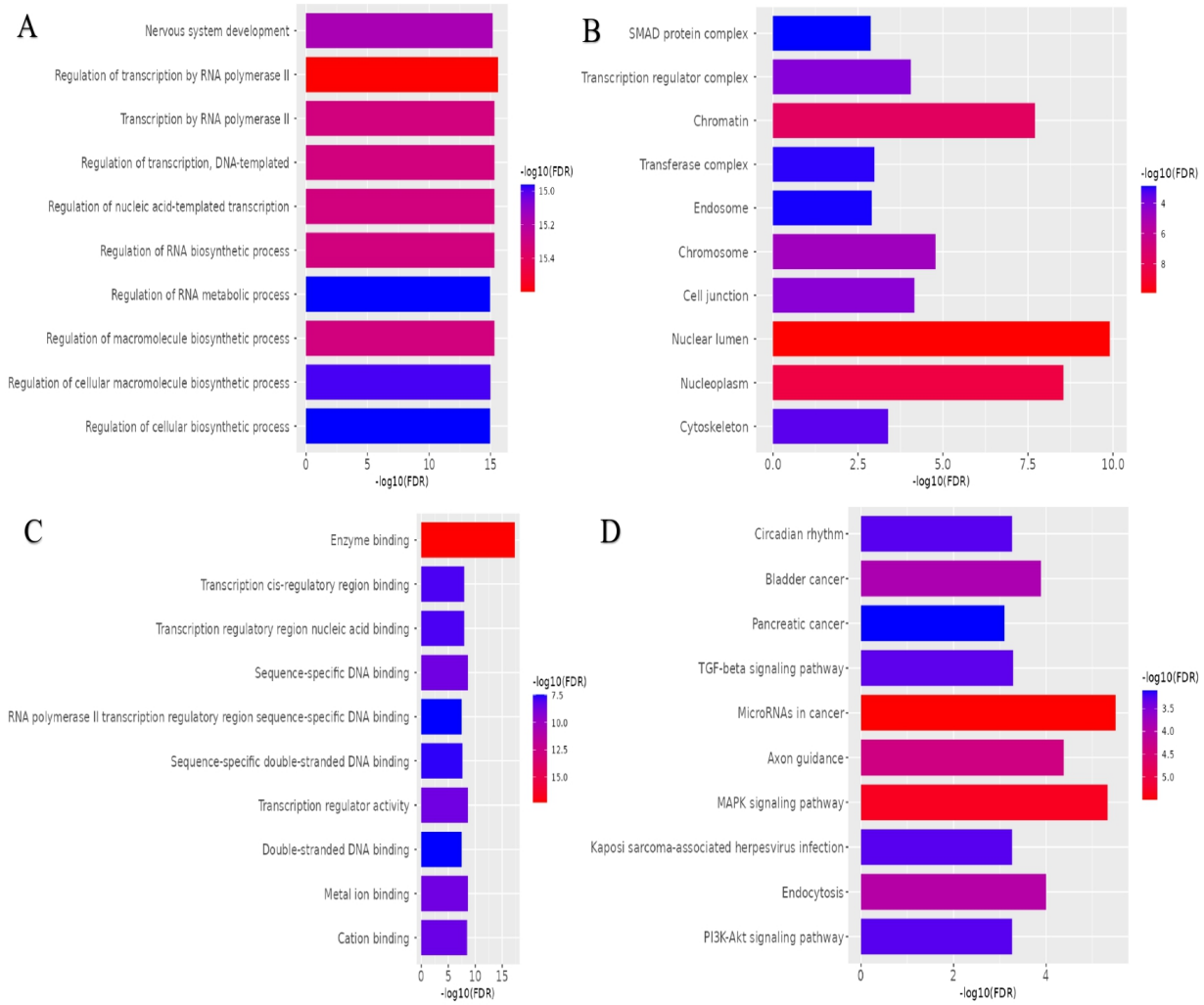

Fig. 5: Go enrichment, A) biological process B) cellular component C) molecular function and D) KEGG

These target genes were also significantly clustered in terms of molecular function, such as enzyme binding (GO:0019899), transcription cis-regulatory region binding (GO:0000976), and transcription regulatory region nucleic acid binding (GO: 0001067) (Fig. 5C).

With regard to KEGG pathway analysis, the 10 significant signaling pathways for the target genes of miR-93-5p were Circadian rhythm, Bladder cancer, Pancreatic cancer, TGF-beta signaling pathway, MicroRNAs in cancer, Axon guidance, MAPK signaling pathway, Kaposi sarcoma-associated herpesvirus infection, Endocytosis, PI3K-Akt signaling pathway (all P and Q values < 0.05) (Fig. 5D). Furthermore, molecular pathways and processes were calculated to generate a PPI network. Relevant PPI were visualized, revealing 879 nodes and 192 edges (Supplementary Fig. 3B; Supplementary Fig. 4). MCODE extracted hub genes from the PPI network where the

most connected genes were SMAD1, SMAD7 and BMPR2. Those 3 genes may be the potential target genes of miR-93-5p and may be involved in the regulatory mechanisms in PCa.

The expression and prognostic validation of the miR-93-5p Target Genes

Three potential target genes of miR-93-5p associated with PCa (SMAD1, SMAD7 and BMPR2) were identified to be downregulated in PCa tissues when compared to controls (Fig. 6A,D,G). The survival analysis showed no significant association between high expression and overall survival (*P*>0.05) (Fig. 6B,E,H).

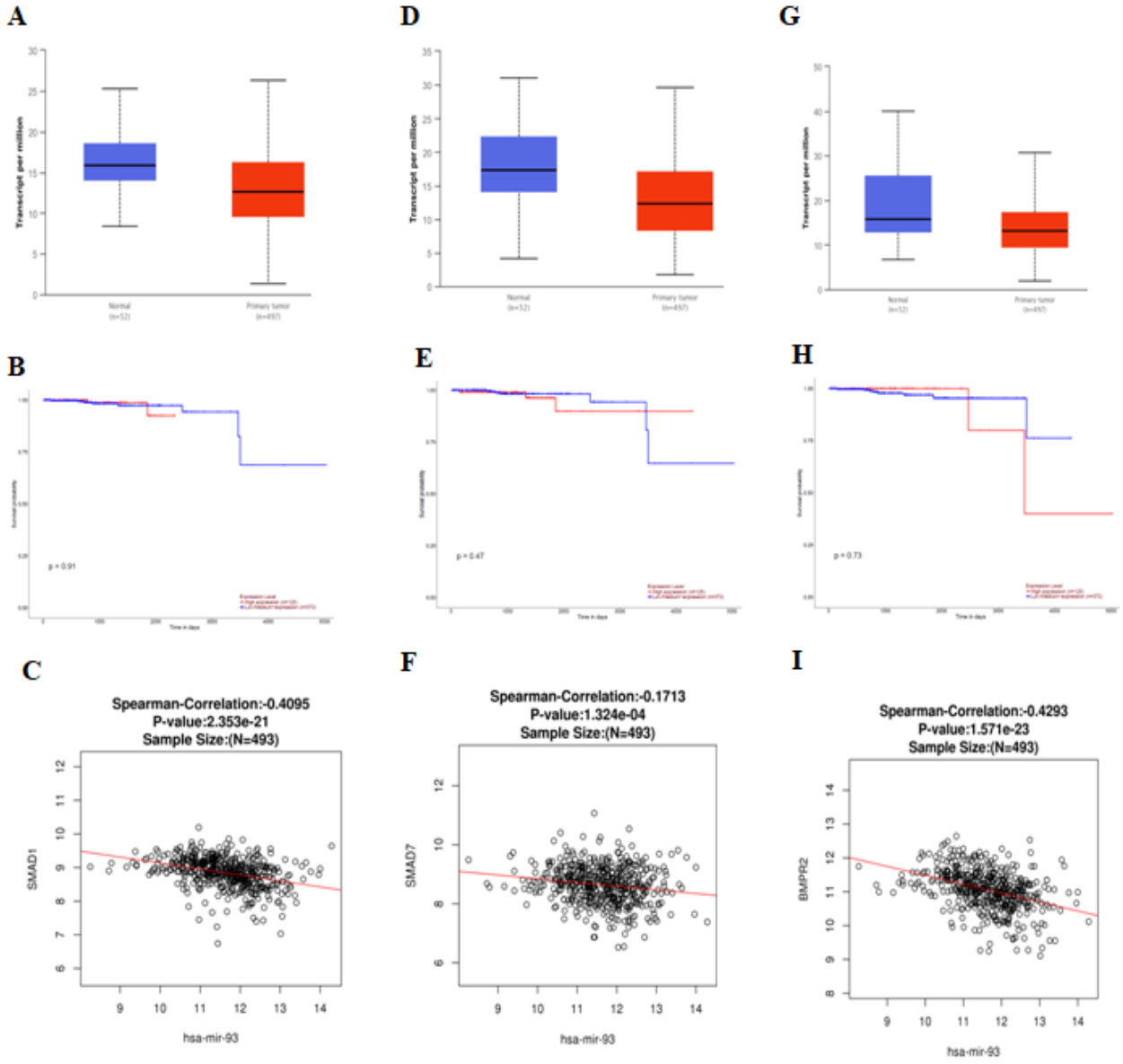

Fig. 6: Validation of the miR-93-5p Target Genes, A) SMAD1 expression, B) KM of SMAD1, C) Spearman correlation between SMAD1 and miR-93-5p, D) S:MAD7 expression, E) KM of SMAD7, F) Spearman correlation between SMAD7 and miR-93-5p, G) BMPR2 expression, H) KM of BMPR2, I) Spearman correlation between BMPR2 and miR-93-5p, KM: Kaplan Meier

Due to miR-93-5p is upregulated in PCa, the genes with decreased expression in PCa are most likely to act as target genes of miR-93-5p. Then Spearman's correlation analysis revealed that SMAD1, SMAD7 and BMPR2 were negatively correlated with miR-93-5p expression in PCa significantly, with $r=-0.409$, $P<0.001$, and $r=-0.171$, $P<0.001$ and $r=-0.429$, $P<0.001$ respectively (Fig. 6C,F,I).

Discussion

The exploration of the molecular mechanism in human cancers could result in the identification of minimally invasive and innovative biomarkers that could improve diagnosis and prediction of the course of the disease. To the best of our knowledge, no comprehensive review so far has been published that provides an overview of the current state of research on miR-93 in PCa.

Then, the current study aimed to investigate the diagnostic and prognostic value of miR-93-5p besides a bioinformatics analysis in the PCa. The meta-analysis forest plots showed that both combined SMD and AUC were 1.26 and 0.84 respectively. This is in line with other studies where miR-93 was markedly upregulated in breast and nasopharyngeal cancers (32,33). Our results indicate that miR-93 may serve as highly accurate diagnostic biomarker that can discern between prostatic cancer and normal tissue.

The pooled hazard ratio (HR) in PCa patients with high miR-93-5p expression of 1.67 is in favor of poorer prognosis which suggests that it may be a useful prognostic marker in PCa. The same finding was stated by Zhang and colleagues who stipulated that patients with high miR-93 expression level had a worse overall survival (34).

In terms of bioinformatics analysis, the top enriched terms of miR-93-5p were TGF-beta signaling, PI3K-Akt and MAPK signaling pathways. The expression of hub PPI target genes SMAD7, SMAD1, and BMPR2 was found to be negatively correlated with miR-93-5p expression levels in PCa patients. However, the survival analysis showed no significant association between high expression of miR-93-5p and overall survival ($P>0.05$).

A meta-analysis on the molecular mechanism and role of miR-93 in a bioinformatics pan-cancer study, without any PCa study included, also found that Smad7 was one of the main hub genes (35). The findings of Hu et al indicate that miR-93-5p regulates the TGF-β1/Smad3 pathway and mediates fibrosis in drug-resistant prolactinoma by targeting Smad7 (36). In hepatocellular carcinoma, miR-93 stimulates cell proliferation, migration, and invasion through the oncogenic c-Met/PI3K/Akt pathway (37), it also promotes cell proliferation through the activation of the latter pathway in gliomas (38). Smad7 has been proven to be targeted by miR-93 in breast and colorectal cancers (39,40).

Furthermore, Smad1 gene is suggested to regulates the growth of PCa by modulating MAPK signals, which limits the mitogenic activity induced by androgens (41), it is considered as a target by suppressing proliferation and invasion of PCa cells (42) and both a potential biomarker and a therapeutic target in drug-resistant multiple myeloma (43).

The gene BMPR2 was discovered to have a strong positive association with recurrence-free survival, and a strong negative association with the occurrence of bone metastasis PCa patients (44).

Actually, functional enrichment analysis provides limited evidence and further validation is needed to determine the role of SMAD1, SMAD7, and BMPR2 in the initiation, development and evolution of PCa.

However, the current study has some limitations: First, few studies were included for the assessment of prognosis ability. Second, the subgroups analyses were not performed, as well as the heterogeneity causes were not evaluated. Third, some means and their standard deviations were not directly obtained from the published papers but extracted from the box plots. Lastly, some eligible studies suffered of the small sample.

Conclusion

miR-93 has significant value in the earlier detection and prognostic assessment of PCa. The findings highlight its potential as a non-invasive biomarker for PCa, besides providing valuable insights into the role of miR-93 in the associated biological pathways. However, more researches with well-designed studies are needed to further understand the complex relationship between miR-93 and PCa.

Journalism Ethics considerations

The authors have fully complied with ethical standards, which encompass a range of issues such as plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, and other relevant concerns.

Acknowledgements

None.

Conflict of interest

The authors declare that they have no competing interest.

Supplementary materials

Available upon request.

References

1. Ferlay J, Colombet M, Soerjomataram I, et al (2021). Cancer statistics for the year 2020: An overview. *Int J Cancer*, 149(4):778–89.
2. Siegel RL, Miller KD, Fuchs HE, Jemal A (2021). Cancer Statistics, *CA Cancer J Clin*, 2021 71(1):7–33.
3. Gasinska A, Jaszczynski J, Rychlik U, Łuczynska E, Pogodzinski M, Palaczynski M (2020). Prognostic Significance of Serum PSA Level and Telomerase, VEGF and GLUT-1 Protein Expression for the Biochemical Recurrence in Prostate Cancer Patients after Radical Prostatectomy. *Pathol Oncol Res*, 26(2):1049–56.
4. Chen D, Cabay RJ, Jin Y, Wang A, Lu Y, Shah-Khan M, et al (2013). MicroRNA Deregulations in Head and Neck Squamous Cell Carcinomas. *J Oral Maxillofac Res*, 4(1): e2.
5. Arantes LMRB, Laus AC, Melendez ME, et al (2017). MiR-21 as prognostic biomarker in head and neck squamous cell carcinoma patients undergoing an organ preservation protocol. *Oncotarget*, 8(6):9911–21.
6. Bartel DP (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, 116(2):281–97.
7. Khan MM, Serajuddin M, Bharadwaj M (2023). Potential plasma microRNAs signature miR-190b-5p, miR-215-5p and miR-527 as non-invasive biomarkers for prostate cancer. *Biomarkers*, 28(2):227-237 .
8. Cao Z, Guo Y, Ao Y, Zhou S (2020). Dysregulated microRNAs in laryngeal cancer: a comprehensive meta-analysis using a robust rank aggregation approach. *Future Oncol*, 16(33):2723–34.
9. Mitchell PS, Parkin RK, Kroh EM, et al (2008). Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA*, 105(30):10513–8.
10. Fendler A, Stephan C, Yousef GM, Kristiansen G, Jung K (2016). The translational potential of microRNAs as biofluid markers of urological tumours. *Nat Rev Urol*, 13(12):734–52.
11. Choi N, Park J, Lee JS, et al (2015). miR-93/miR-106b/miR-375-CIC-CRABP1: a novel regulatory axis in prostate cancer progression. *Oncotarget*, 6(27):23533–47.
12. Ciszkowicz E, Porzycki P, Semik M, Kaznowska E, Tyrka M (2020). MiR-93/miR-375: Diagnostic Potential, Aggressiveness Correlation and Common Target Genes in Prostate Cancer. *Int J Mol Sci*, 21(16):5667.
13. Liu JJ, Zhang X, Wu XH (2018). miR-93 Promotes the Growth and Invasion of Prostate Cancer by Upregulating Its Target Genes TGFBR2, ITGB8, and LATS2. *Mol Ther Oncolytics*, 11:14–9.
14. Yang K, Gao ZY, Li TQ, et al (2019). Antitumor activity and the mechanism of a green tea (Camellia sinensis) polysaccharide on

prostate cancer. *Int J Biol Macromol*, 122:95–103.
15. Zedan AH, Osther PJS, Assenholt J, Madsen JS, Hansen TF (2020). Circulating miR-141 and miR-375 are associated with treatment outcome in metastatic castration resistant prostate cancer. *Sci Rep*, 10(1):227.
16. Martínez-González LJ, Sánchez-Conde V, González-Cabezuelo JM, et al (2021). Identification of MicroRNAs as Viable Aggressiveness Biomarkers for Prostate Cancer. *Biomedicines*, 9(6):646.
17. Zhang S, Liu C, Zou X, et al (2021). MicroRNA panel in serum reveals novel diagnostic biomarkers for prostate cancer. *PeerJ*, 9:e11441.
18. Page MJ, McKenzie JE, Bossuyt PM, et al (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*, 372:n71.
19. Rohatgi A (2022). WebPlotDigitizer User Manual Version 4.6. *https://automeris.io/WebPlotDigitizer/userManual.pdf*
20. Stang A (2010). Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol,* 25(9):603–5.
21. Liu W, Wang X (2019). Prediction of functional microRNA targets by integrative modeling of microRNA binding and target expression data. *Genome Biol*, 20(1):18.
22. Agarwal V, Bell GW, Nam JW, Bartel DP (2015). Predicting effective microRNA target sites in mammalian mRNAs. *Elife*, 4:e05005.
23. Backes C, Kehl T, Stöckel D, et al (2017). miRPathDB: a new dictionary on microRNAs and target pathways. *Nucleic Acids Res*, 45(Database issue):D90–6.
24. Thomas PD (2017). The Gene Ontology and the Meaning of Biological Function. *Methods Mol Biol*, 1446:15–24.
25. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K (2017). KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res,* 45(D1):D353–61.
26. Bader GD, Hogue CW (2003). An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics*, 4(1):2.
27. Chandrashekar DS, Bashel B, Balasubramanya SAH, et al (2017). UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia*, 19(8):649–58.
28. Vasaikar SV, Straub P, Wang J, Zhang B (2018). LinkedOmics: analyzing multi-omics data within and across 32 cancer types. *Nucleic Acids Res*, 46(D1):D956–63.
29. Barceló M, Castells M, Pérez-Riba M, Bassas L, Vigués F, Larriba S (2020). Seminal plasma microRNAs improve diagnosis/prognosis of prostate cancer in men with moderately altered prostate-specific antigen. *Am J Transl Res*, 12(5):2041–51.
30. Porras-Quesada P, González-Cabezuelo JM, Sánchez-Conde V, et al (2022). Role of IGF2 in the Study of Development and Evolution of Prostate Cancer. *Front Genet*, 12:740641.
31. Wang C, Tian S, Zhang D, et al (2020). Increased expression of microRNA-93 correlates with progression and prognosis of prostate cancer. *Medicine (Baltimore)*, 99(22):e18432.
32. Deng Z qun, Qian J, Liu F qiong, et al (2014). Expression level of miR-93 in formalin-fixed paraffin-embedded tissues of breast cancer patients. *Genet Test Mol Biomarkers*, 18(5):366–70.
33. Lyu X, Fang W, Cai L, et al (2014). TGFβR2 is a major target of miR-93 in nasopharyngeal carcinoma aggressiveness. *Mol Cancer*, 13:51.
34. Zhang H, Zhang J, Meng F, et al (2019). MicroRNA-93 promotes the tumorigenesis of osteosarcoma by targeting TIMP2. *Biosci Rep*, 39(8):BSR20191237.
35. Gao Y, Deng K, Liu X, et al (2019). Molecular mechanism and role of microRNA-93 in human cancers: A study based on bioinformatics analysis, meta-analysis, and quantitative polymerase chain reaction validation. *J Cell Biochem*, 120(4):6370–83.
36. Hu B, Mao Z, Du Q, et al (2019). miR-93-5p targets Smad7 to regulate the transforming growth factor-β1/Smad3 pathway and mediate fibrosis in drug-resistant prolactinoma. *Brain Res Bull*, 149:21–31.
37. Katsuya O, Hiromitsu H, Jinhua W, et al (2015). MicroRNA-93 activates c-Met/PI3K/Akt pathway activity in hepatocellular carcinoma by directly inhibiting PTEN and CDKN1A. *Oncotarget*, 6(5):3211-3224.
38. Jiang L, Wang C, Lei F, et al (2015). miR-93 Promotes Cell Proliferation in Gliomas

through Activation of PI3K/Akt Signaling Pathway. *Oncotarget*, 6(10):8286–99.
39. Smith AL, Iwanaga R, Drasin DJ, et al (2012). The miR-106b-25 cluster targets Smad7, activates TGF-β signaling, and induces EMT and tumor initiating cell characteristics downstream of Six1 in human breast cancer. *Oncogene*, 31(50):5162–71.
40. Tang Q, Zou Z, Zou C, et al (2015). MicroRNA-93 suppress colorectal cancer development via Wnt/β-catenin pathway downregulating. *Tumor Biol*, 36(3):1701–10.
41. Qiu T, Grizzle WE, Oelschlager DK, Shen X, Cao X (2007). Control of prostate cell growth: BMP antagonizes androgen mitogenic activity with incorporation of MAPK signals in Smad1. *EMBO J*, 26(2):346–57.
42. Qu F, Zheng J, Gan W, et al (2017). MiR-199a-3p suppresses proliferation and invasion of prostate cancer cells by targeting Smad1. *Oncotarget*, 8(32):52465–73.
43. Wu J, Zhang M, Faruq O, et al (2021). SMAD1 as a biomarker and potential therapeutic target in drug-resistant multiple myeloma. *Biomark Res*, 9(1):48.
44. Kobayashi A, Okuda H, Xing F, et al (2011). Bone morphogenetic protein 7 in dormancy and metastasis of prostate cancer stem-like cells in bone. *J Exp Med*, 208(13):2641–55.