

Metastasis-associated protein I rewires prostate cancer transcriptomes via direct RNA interactions governing gene expression and alternative splicing

Guanlin Qu, Fuhao Li , Bin Wu, Enlai Li, Shen Xu and Lei Chen 

Abstract

Objective: This study investigated whether metastasis-associated protein I (MTAI) acts as an RNA-binding protein to directly regulate gene expression and alternative splicing in prostate cancer, thereby exploring its dual transcriptional and post-transcriptional roles.

Methods: MTAI-stable-knockdown PC-3 cells were subjected to RNA sequencing and formaldehyde RNA immunoprecipitation sequencing to characterize the MTAI–RNA interactome and its functional significance.

Results: MTAI knockdown dysregulated 1248 genes and 2367 alternative splicing events. Differentially expressed genes were enriched in extracellular matrix remodeling, PI3K–Akt signaling, and hypoxia-response pathways, whereas alternatively spliced genes were associated with spliceosome and RNA-processing pathways. Formaldehyde RNA immunoprecipitation sequencing confirmed MTAI binding to GC-rich RNA motifs, with significant overlap between MTAI-bound targets and dysregulated genes or splicing events. Representative findings included MTAI binding to the 3′ untranslated region of COL6A1, resulting in its downregulation, and regulation of SNHG17 splicing, indicating direct roles in RNA stability and splicing regulation.

Conclusion: MTAI functions as both an epigenetic modulator and an RNA-binding protein in prostate cancer, directly regulating RNA networks that promote tumor progression. These findings reveal novel RNA-mediated oncogenic mechanisms and highlight the therapeutic potential of MTAI in prostate cancer.

Corresponding author:

Lei Chen, Second Affiliated Hospital of Anhui Medical University Department of Urology, No. 678, Furong Road, Shushan District, Hefei, 230601, China.

Email: chenlei0718@sina.com.cn

Department of Urology, The Second Affiliated Hospital of Anhui Medical University, China



Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative

Commons Attribution-NonCommercial 4.0 License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (<https://us.sagepub.com/en-us/nam/open-access-at-sage>).

Keywords

Metastasis-associated protein 1, RNA-binding protein, alternative splicing, prostate cancer, RNA stability, formaldehyde RNA immunoprecipitation sequencing, transcriptome

Date received: 5 October 2025; accepted: 5 May 2026

Introduction

Metastasis-associated protein 1 (MTA1), a well-established epigenetic regulator, is widely implicated in cancer progression through its chromatin-remodeling activities.^{1–7} However, its role in post-transcriptional RNA-mediated mechanisms remains largely unexplored, particularly in prostate cancer (PCa), a leading cause of cancer-related mortality.^{8–10} Although MTA1 is known to drive oncogenic pathways via transcriptional regulation, emerging evidence suggests that RNA-binding proteins play critical roles in shaping cancer transcriptomes by modulating RNA stability, translation, and alternative splicing.¹¹ However, whether MTA1 operates as an RNA-binding protein (RBP) to directly govern RNA networks in PCa pathogenesis remains unclear.

Alternative splicing, a process enabling a single gene to generate multiple protein isoforms, plays a pivotal role in cancer development and progression.¹² Dysregulation of splicing mechanisms is frequently observed in tumors, leading to the production of aberrant isoforms that promote oncogenic traits such as uncontrolled proliferation, evasion of apoptosis, and metastasis. For example, splice variants of genes such as TP53, BCL2L1, or CD44 may lose tumor-suppressive functions or gain pro-survival properties.^{13–15} Additionally, mutations in splicing factors or altered expression of splicing regulators are directly linked to cancer pathogenesis.^{16–18} These disruptions can activate oncogenic pathways, enhance angiogenesis, or confer therapy resistance.¹⁹ Targeting aberrant

splicing events or the splicing machinery is emerging as a novel therapeutic strategy in precision oncology.^{18,20}

This study reveals MTA1's dual chromatin/RBP role in PCa via RNA sequencing (RNA-seq) and formaldehyde RNA immunoprecipitation sequencing (fRIP-seq). MTA1 knockdown dysregulated 1248 genes and 2367 splicing events, affecting extracellular matrix (ECM), PI3K–Akt, and hypoxia pathways. It directly binds GC-rich RNA motifs, with significant overlap between MTA1-bound targets and altered genes/splicing events, validated by splicing ratio analysis. MTA1 bridges transcriptional and post-transcriptional regulatory networks, highlighting its therapeutic potential in RNA-centric oncogenesis.²¹

Materials and methods

Cloning and plasmid construction

The lentiviral stock solution was purchased from Gemma (Suzhou, China). The siRNA targeting MTA1 (siMTA1) sequence was 5'-GGAGAGATTCGAGTAGGAAAC-3' (sense).

Cell culture and transfections

The prostate cancer-3 (PC-3) cell line (Procell Life Science & Technology Co., Ltd.; China) was cultured at 37°C with 5% carbon dioxide (CO₂) in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) supplemented with 10% fetal bovine serum, 100 µg/mL streptomycin, and 100 U/mL penicillin. The lentiviral stock solution was purchased from

GenePharma. PC-3 cells were infected with the lentiviral particles. Seventy-two hours post-transfection, cells were selected with 5 µg/mL puromycin, and stable clones were maintained in medium containing 2 µg/ml puromycin.

Assessment of gene expression

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal control gene for assessing the effects of MTA1 knockdown. cDNA synthesis was performed using standard procedures, and reverse transcription quantitative polymerase chain reaction (PCR) (RT-qPCR) was carried out on a Bio-Rad S1000 system using Hieff qPCR SYBR® Green Master Mix (Low Rox Plus; YEASEN; China). Primer sequences are provided in Additional file 1. Relative transcript levels were normalized to GAPDH using 2- $\Delta\Delta$ CT method. Comparisons were performed using a paired student's t-test in GraphPad Prism software (San Diego, CA, USA).

Western blot

Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer, and proteins (30 µg) were separated by 8%–10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore). After blocking, membranes were incubated with appropriately diluted primary antibodies, followed by horseradish peroxidase (HRP)-conjugated secondary antibodies. Protein bonds were visualized using an enhanced chemiluminescence (ECL) detection system (Thermo Fisher Scientific).

RNA extraction and sequencing

Total RNA was extracted from PC-3 cells using TRIzol Reagent (Invitrogen, cat. NO 15596026) following the method described

by Chomczynski. DNA digestion was carried out after RNA extraction by DNaseI. RNA quality was assessed by Absorbance260/Absorbance280 ratio using a Nanodrop™ OneC spectrophotometer (Thermo Fisher Scientific Inc.). RNA Integrity was confirmed by 1.5% agarose gel electrophoresis. Qualified RNA samples were quantified using a Qubit 3.0 fluorometer with the Qubit™ RNA Broad Range Assay Kit (Life Technologies, Q10210). A total of 2 µg RNA was used for stranded RNA-seq library preparation using the KCTM Stranded mRNA Library Prep Kit for Illumina (Catalog No. DR08402, Wuhan Seqhealth Co., Ltd.; China), following the manufacturer's instructions. PCR products corresponding to 200–500 bp were enriched, quantified, and sequenced on the MGISEQ-T7 platform (MGI) using a PE150 sequencing model.

RNA-seq raw data processing and alignment

Raw reads containing more than two ambiguous bases were discarded. Adapter sequences and low-quality bases were trimmed from raw reads using FASTX-Toolkit (Version 0.0.13). Reads shorter than 16 nucleotides (nt) were also removed. Clean reads were then aligned to the GRCh38 human reference genome using HISAT2,²² allowing up to four mismatches. Uniquely mapped reads were used for gene-level read counting and fragments per kilobase of transcript per million fragments mapped (FPKM) calculation.²³

Differentially expressed gene (DEG) analysis

The R Bioconductor package 'DESeq2' was used to identify DEGs.²⁴ Adjusted P-value <0.05 and fold change >2 or <0.5 were set as the thresholds for DEG identification.

Alternative splicing analysis

Alternative splicing events (ASEs) and regulated alternative splicing events (RASEs) between samples were identified and quantified using the ABLas pipeline as previously described. In brief, ABLas detects 10 types of ASEs based on splice junction reads, including exon skipping (ES), alternative 5' splice site (A5SS), alternative 3' splice site (A3SS), mutually exclusive exons (MXE), mutually exclusive 5' untranslated regions (UTRs) (5pMXE), mutually exclusive 3' UTRs (3pMXE), cassette exons, A3SS&ES, and A5SS&ES. To assess RBP-regulated ASEs, student's t-test was used to evaluate the significance of AS ratio changes. Events with P-values corresponding to a false discovery rate (FDR) <0.05 were considered RBP-regulated ASEs.

Functional enrichment analysis (RNA-seq)

To identify functional categories of DEGs, Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were analyzed using the KOBAS 2.0 server (19). A hypergeometric test and Benjamini–Hochberg FDR correction were applied to determine the enrichment significance of each term.

Co-immunoprecipitation and library preparation

PC-3 cells were cross-linked with 1% formaldehyde, quenched with 0.125 M glycine, and lysed in ice-cold buffer containing RNase/protease inhibitors. Lysates were treated with RQ1 DNase and subjected to MNase digestion. Immunoprecipitation (IP) was performed using an anti-MTA1 antibody or control immunoglobulin G (IgG), followed by binding to protein A/G magnetic beads. Beads were washed sequentially with lysis buffer, high-salt buffer, and polynucleotide kinase (PNK) buffer. Complexes were eluted at 70°C, digested

with Proteinase K, and RNA was extracted using TRIzol. Sequencing libraries were prepared using the KAPA RNA HyperPrep Kit and sequenced on an Illumina NovaSeq platform (150 bp paired-end).

Data analysis

After alignment with HISAT2 and removal of PCR duplicates, peaks were called using Piranha and ABLIRC (5 bp sliding window; 8 consecutive windows with $\geq 2.5\times$ depth or median depth >50 ; termination threshold $<4\%$ of maximum peak depth). Peaks with $P < 0.05$ or maximum depth ≥ 10 (based on $500\times$ randomization) were retained. Peaks showing ≥ 4 -fold enrichment input were selected as final peaks. Target genes were annotated, and binding motifs were identified using Hypergeometric Optimization of Motif EnRichment (HOMER).

Functional enrichment analysis (fRIP-seq)

To identify functional categories of peak-associated genes (target genes), GO terms and KEGG pathway enrichment were analyzed using KOBAS 2.0 server.²⁵ A hypergeometric test and Benjamini–Hochberg FDR correction were applied to determine enrichment significance of each term.

Results

MTA1 knockdown suppresses proliferation but does not induce apoptosis in PC-3 cells

To assess the functional impact of MTA1 in PCa progression, we generated stable MTA1-knockdown PC-3 cells using shRNA. RT-qPCR confirmed a significant reduction in MTA1 mRNA levels compared with negative control (NC) cells (Figure 1(a)), and western blot analysis validated near-complete ablation of MTA1

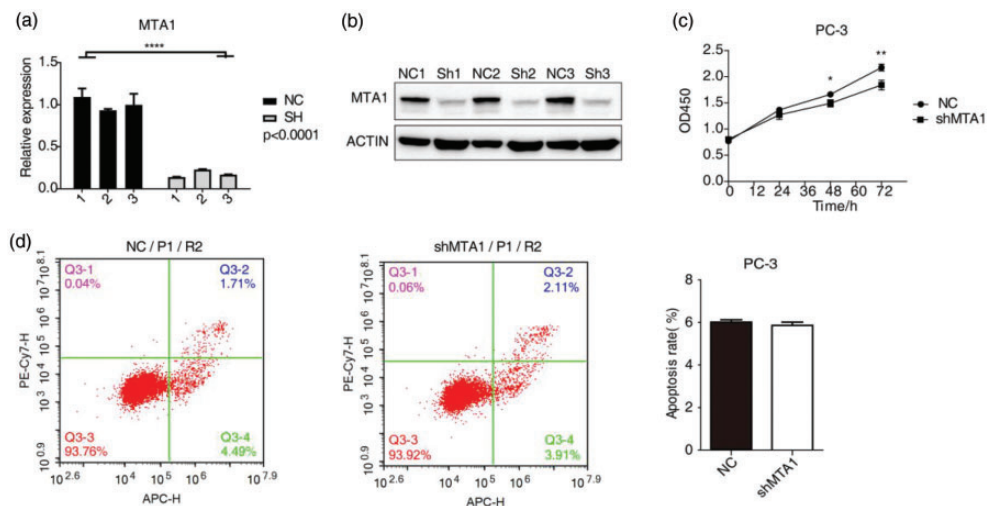


Figure 1. MTA1 knockdown inhibited PC-3 cell proliferation. (a) Bar plot showing RT-qPCR results of NC and shMTA1 samples ($P < 0.0001$); (b) decreased MTA1 expression was confirmed by western blot assay. (c) The effect of MTA1 knockdown on PC-3 cell proliferation was detected by CCK-8 assay. (d) The effect of MTA1 knockdown on PC-3 apoptosis was detected by Annexin V–Alexa Fluor 647/PI flow cytometric apoptosis assay. MTA1: metastasis-associated protein 1; PC-3: prostate cancer-3; NC: negative control; shMTA1: MTA1 knockdown; RT-qPCR: reverse transcription quantitative polymerase chain reaction; CCK-8: cell counting Kit-8; PI: propidium iodide.

protein expression (Figure 1(b)). These results confirm the efficiency of MTA1 silencing and provide a reliable model for phenotypic analysis.

Functional assays revealed that MTA1 depletion markedly impaired cell proliferation. Cell counting kit-8 (CCK-8) assays demonstrated a time-dependent decline in cell viability, with proliferative capacity reduced by approximately 60% at 72 h post-knockdown (Figure 1(c)). In contrast, Annexin V–Alexa Fluor 647/propidium iodide (PI) staining showed no statistically significant increase in apoptotic cells between NC and shMTA1 groups (Figure 1(d)). Although early (Annexin V+/PI–) and late (Annexin V+/PI+) apoptotic populations showed minor fluctuations, these changes were not significant, suggesting that MTA1 knockdown primarily disrupts proliferative pathways without triggering apoptosis in PC-3 cells under these experimental conditions.

Transcriptomic profiling reveals large-scale gene expression dysregulation upon MTA1 knockdown

RNA-seq analysis was performed to investigate the global transcriptional impact of MTA1 depletion in PC-3 cells. Comparative analysis between NC and MTA1-knockdown (shMTA1) groups identified 1248 DEGs (Figure 2(c)). A bar plot summarizing RNA-seq data demonstrated strong reproducibility among biological replicates within each group (Figure 2(a)), supporting the reliability of downstream analyses.

Principal component analysis (PCA) based on FPKM values showed clear separation between NC and shMTA1 samples (Figure 2(b)), with confidence ellipses indicating distinct transcriptomic profiles. The first two principal components accounted for more than 70% of the total variance, underscoring the substantial effect of MTA1 on gene expression programs.

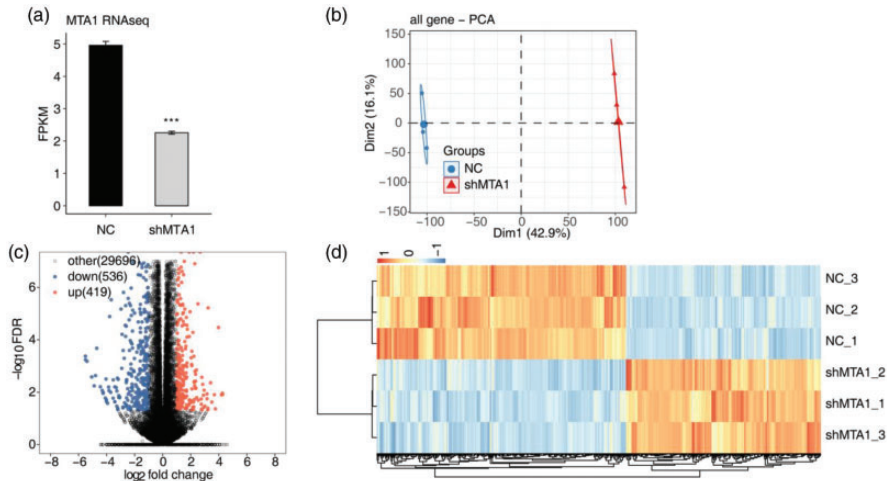


Figure 2. RNA-seq after MTA1 knockdown in PC-3 cells. (a) Bar plot showing the RNA-seq results of NC and shMTA1 samples; (b) PCA based on FPKM values of all detected genes. The ellipse for each group is the confidence ellipse. (c) Volcano plot showing all DEGs (e.g. VEGFA, MYC, CDKN1A, and TIMP3) between NC and shMTA1 samples using DESeq2. Adjusted P-value <0.05 and fold change ≥ 2 or ≤ 0.5 were used as thresholds. (d) Hierarchical clustering heat map showing expression levels of all DEGs. MTA1: metastasis-associated protein 1; PC-3: prostate cancer-3; NC: negative control; shMTA1: MTA1 knockdown; RNA-seq: RNA sequencing; PCA: principal component analysis; FPKM: fragments per kilobase of transcript per million mapped reads; DEGs: differentially expressed genes; DESeq2: differential expression analysis for sequence count data (R package).

Volcano plot visualization (Figure 2(c)) further illustrated DEG distribution, with up-regulated genes enriched in tumor-suppressive pathways (e.g. ECM remodeling) and down-regulated genes associated with oncogenic signaling (e.g. PI3K–Akt pathway).

Hierarchical clustering of all DEGs (Figure 2(d)) demonstrated coherent expression patterns across replicates, with distinct clusters segregating NC and shMTA1 groups. Notably, key oncogenes (VEGFA and MYC) were suppressed, whereas tumor suppressors (CDKN1A and TIMP3) were upregulated, reflecting MTA1's dual role as a transcriptional activator and repressor. These findings position MTA1 as a master regulator of PCa transcriptomes, orchestrating both pro-tumorigenic and anti-tumorigenic networks.

The large-scale gene expression dysregulation observed in MTA1-depleted cells

underscores its central role in maintaining oncogenic transcriptional programs. Enrichment of ECM-related genes among DEGs aligns with MTA1's reported role in metastasis, whereas suppression of PI3K–Akt signaling highlights its involvement in survival pathways. The PCA and clustering results collectively validate the robustness of the RNA-seq dataset, providing a foundation for mechanistic exploration of MTA1's chromatin and RNA-binding activities in subsequent analyses.

Functional annotation of MTA1-regulated genes in PC-3 cells

To delineate the biological significance of MTA1-dependent transcriptional changes, we performed functional enrichment analysis of upregulated and downregulated DEGs. For upregulated DEGs, GO terms

were enriched in ECM organization, collagen fibril assembly, and hypoxia responses (Figure 3(a)), whereas KEGG pathway analysis highlighted ECM–receptor interaction and focal adhesion (Figure 3(b)). Key upregulated genes, including COL6A1 and LOXL2, showed significant expression increases (Figure 3(c)), suggesting that MTA1 knockdown modulates ECM-related tumor–stroma crosstalk.

Downregulated DEGs were enriched in oncogenic pathways, with GO terms encompassing PI3K–Akt signaling, cell

proliferation, and angiogenesis (Figure 3 (d)). KEGG pathway analysis further linked these genes to “Proteoglycans in cancer” and “HIF-1 signaling” (Figure 3(e)). Notably, VEGFA and MYC showed marked suppression (Figure 3(f)), consistent with MTA1’s role in sustaining proliferative and pro-survival networks.

The dichotomy between upregulated ECM/hypoxia-related genes and downregulated proliferative/angiogenic factors underscores MTA1’s dual regulatory capacity. Although MTA1 loss disrupts oncogenic

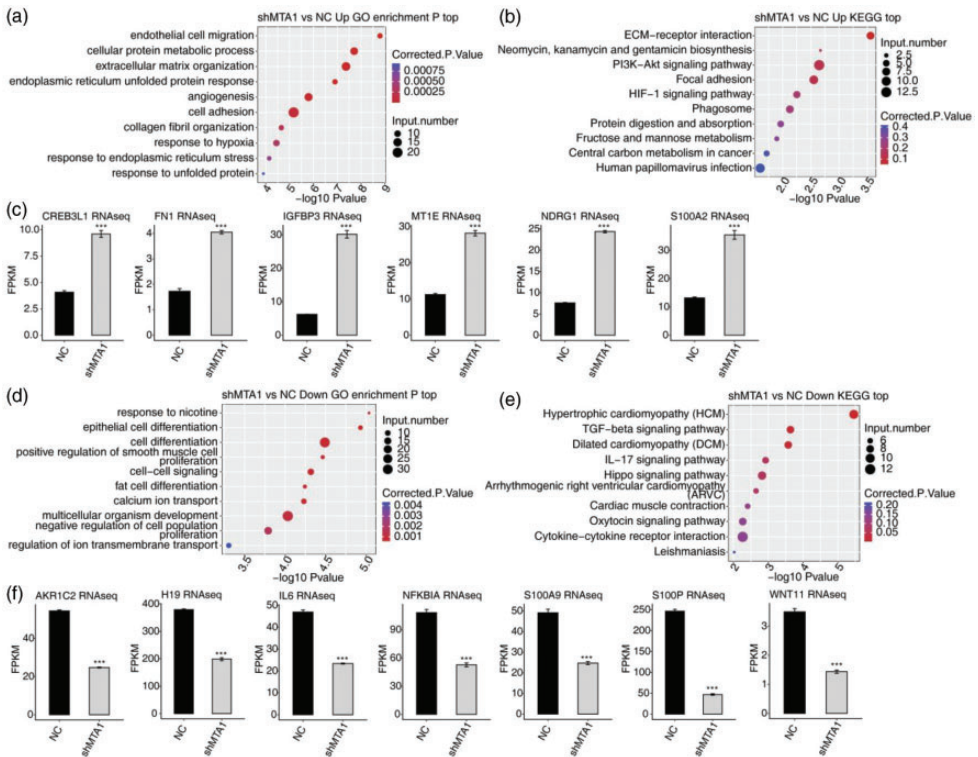


Figure 3. MTA1 regulates expression of genes in PC-3 cells. (a and b) Bubble diagram showing the most enriched GO biological processes and KEGG pathways among upregulated DEGs. (c) Bar plot showing expression patterns and statistical differences of top upregulated DEGs. Error bars represent mean ± SEM. ***P-value < 0.001. (d and e) Bubble diagram showing the most enriched GO biological processes and KEGG pathways among downregulated DEGs. (f) Bar plot showing expression patterns and statistical differences of top downregulated DEGs. Error bars represent mean ± SEM. ***P-value < 0.001. MTA1: metastasis-associated protein 1; PC-3: prostate cancer-3; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; DEGs: differentially expressed genes; Up DEGs: upregulated differentially expressed genes; Down DEGs: downregulated differentially expressed genes; SEM: standard error of the mean.

signaling, it concurrently activates compensatory ECM remodeling, potentially reflecting adaptive stress responses in PC-3 cells. The prominence of COL6A1 and VEGFA highlights MTA1's influence on tumor microenvironment dynamics, aligning with its established role in metastasis. These findings position MTA1 as a transcriptional rheostat balancing pro-tumorigenic signaling and stromal interactions in PCa progression.

MTA1 governs alternative splicing networks in PC-3 cells

MTA1 knockdown in PC-3 cells induced widespread dysregulation of AS, with 2367 significant RASEs identified across multiple splicing types. ES was the most prevalent event, followed by alternative 3' splice site and 5' splice site usage (Figure 4(a)). Functional enrichment analysis of genes associated with these RASEs (RASGs) revealed strong associations with spliceosome assembly, RNA processing, and mRNA surveillance pathways (Figure 4 (b)), positioning MTA1 as a key regulator of post-transcriptional RNA maturation.

A Venn analysis showed that 18% of RASGs overlapped with DEGs (Figure 4 (c)), suggesting coordinated transcriptional and splicing regulation by MTA1. For instance, IL6 exhibited ES (Figure 4(d), left panel), with MTA1 depletion reducing inclusion of exon 2 (Figure 4(d), right panel). Similarly, TNFRSF12A showed altered 3' splice site selection (Figure 4(e)), leading to a truncated isoform associated with apoptotic resistance. Integrative genomics viewer (IGV) sashimi plots and schematic diagrams validated these events, with RNA-seq data confirming significant shifts in splicing ratios.

The overlap between RASGs and DEGs suggests that MTA1 couples transcriptional output with splicing regulation, potentially through direct RNA binding or

chromatin-mediated coordination. Enrichment of spliceosome-related pathways among RASGs underscores MTA1's role in maintaining RNA processing machinery integrity. The IL6 and TNFRSF12A examples highlight how MTA1-driven splicing alterations may influence cytokine signaling and survival pathways, respectively, contributing to PCa progression. These findings extend MTA1's functional repertoire beyond chromatin remodeling, positioning it as a critical node in both transcriptional and post-transcriptional oncogenic networks.

Large-scale gene expression dysregulation profiling of MTA1–RNA interactions via fRIP-seq

To map direct RNA targets of MTA1, fRIP-seq was performed in PC-3 cells. Western blot analysis confirmed efficient immunoprecipitation of MTA1 with minimal contamination from control IgG (Figure 5(a)). Sequencing reads showed a broad genomic distribution, with peaks predominantly localized to protein-coding regions (Figure 5(b)). Pie chart analysis further revealed that 42% of peaks were located in exons, followed by 3' UTRs (28%) and introns (20%) (Figure 5 (c) to (d)), suggesting that MTA1 preferentially binds mature transcripts. Overlap analysis between biological replicates identified approximately 80% shared peaks (Figure 5e), indicating strong experimental reproducibility.

HOMER motif analysis uncovered GC-rich sequences as the top MTA1-binding motifs (Figure 5f), consistent with a potential role in stabilizing structured RNA regions. IGV sashimi plots validated MTA1 interactions at specific loci, including RPL32 and SNHG10 (Figure 5(g) to (h)). For RPL32, MTA1 binding clustered near exon–intron junctions, whereas SNHG10 exhibited peaks across spliced exons, consistent with MTA1 enrichment in exonic regions.

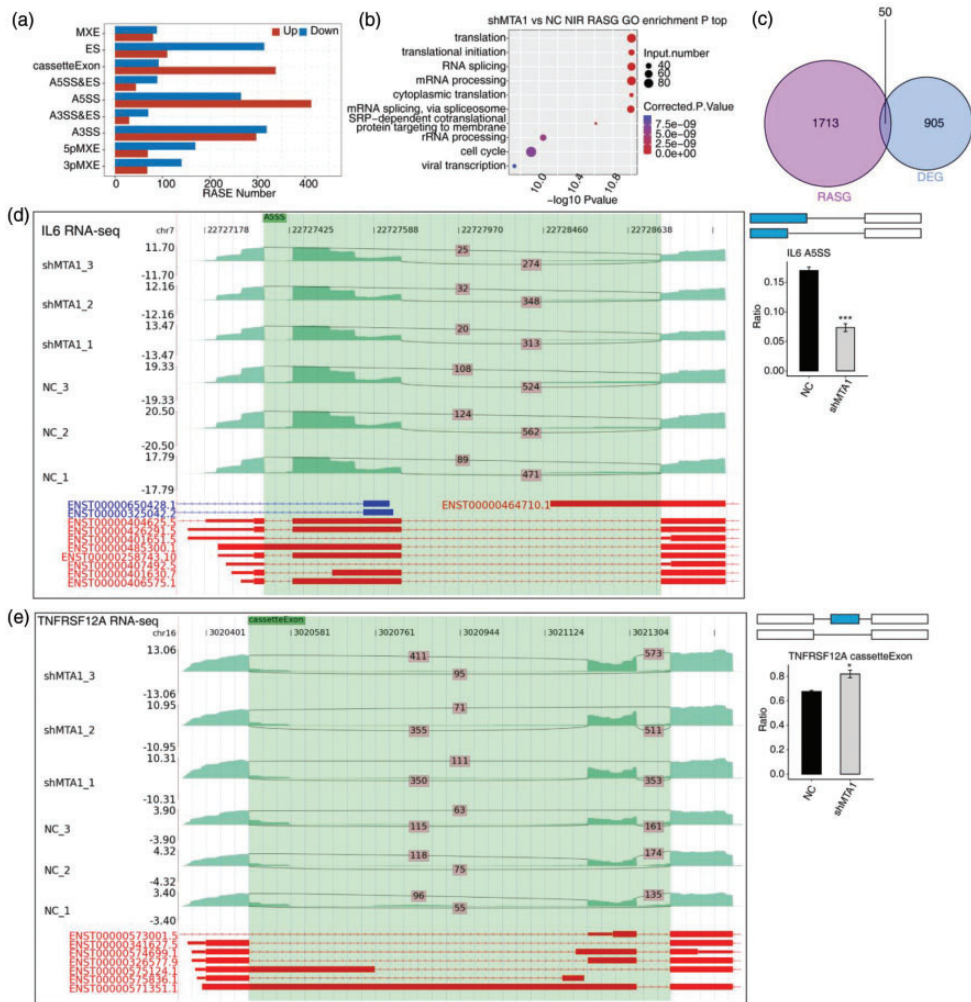


Figure 4. MTA1 regulates gene alternative splicing in PC-3 cells. (a) Bar plot showing the number of significant RASEs. X-axis: number of RASEs. Y-axis: types of AS events. (b) Bubble diagram showing the most enriched GO biological processes among RASGs. (c) Venn diagram showing overlap between RASGs and DEGs. (d to f) MTA1 regulates alternative splicing of IL6 (e) and TNFRSF12A (f). Left panel: IGV sashimi plot showing regulated alternative splicing events and binding sites across mRNA. Read distribution of RASEs is shown in the upper panel, and gene structures are shown below. Right panel: Schematic diagrams depicting exon/intron structures of ASEs. RNA-seq validation of ASEs is shown at the bottom of the right panel. Error bars represent mean $\pm$ SEM. ***P-value < 0.001, **P-value < 0.01, *P-value < 0.05. MTA1: metastasis-associated protein 1; PC-3: prostate cancer-3; RASEs: regulated alternative splicing events; RASGs: regulated alternative splicing genes; GO: Gene Ontology; DEGs: differentially expressed genes; AS: alternative splicing; ASEs: alternative splicing events; IGV: Integrative Genomics Viewer; SEM: standard error of the mean; IL6: interleukin 6; TNFRSF12A: tumor necrosis factor receptor superfamily member 12A; mRNA: messenger RNA; RNA-seq: RNA sequencing.



Figure 5. Characterization of the MTAI–RNA interaction profile by fRIP-seq. (a) WB experiment to verify IP efficiency; (b) bar plot showing read distribution across the reference genome; (c and d) Pie charts showing peak distribution across the reference genome; (e) venn diagram showing overlapping peaks between two IP samples; (f) motif analysis showing the top five peaks preferred MTAI-binding motifs identified using HOMER software; (g and h) IGV sashimi plots showing target peak genes, RPL32 (g) and SNHG10 (h). Read distribution is shown in the upper panel, and gene transcripts are shown below. MTAI: metastasis-associated protein I; fRIP-seq: formaldehyde RNA immunoprecipitation sequencing; WB: western blot; IP: immunoprecipitation; HOMER: Hypergeometric Optimization of Motif EnRichment; IGV: Integrative Genomics Viewer; RPL32: ribosomal protein L32; SNHG10: small nucleolar RNA host gene 10.

The exon-centric binding profile and GC-rich motif preference suggest that MTA1 may regulate RNA stability, splicing, or translation. Its interaction with RPL32 implies a role in ribosome biogenesis, whereas SNHG10 binding highlights potential involvement in non-coding RNA networks. The overlap of peaks across replicates and motif conservation reinforce MTA1's specificity as an RBP, bridging its epigenetic and post-transcriptional functions in PCA progression.

Dual regulatory roles of MTA1 in RNA stabilization and splicing

MTA1 orchestrates prostate cancer progression through dual mechanisms of RNA stabilization and splicing regulation. Integration of fRIP-seq and RNA-seq data identified 312 DEGs overlapping with MTA1-bound RNAs (Figure 6(a)), directly linking its RNA-binding activity to

transcriptional output. A key example is COL6A1, a collagen gene critical for ECM remodeling. fRIP-seq localized MTA1 binding to the 3' UTR of COL6A1 (Figure 6(b)), whereas RNA-seq showed its significant downregulation following MTA1 knockdown (Figure 6(c)). These findings suggest that MTA1 stabilizes COL6A1 transcripts via 3' UTR interactions, thereby maintaining ECM integrity and supporting tumor microenvironment adaptation.

Beyond transcriptional control, MTA1 directly modulates alternative splicing by binding splice-proximal regions. Approximately 22% of RASGs overlapped with its RNA targets (Figure 7(a)), exemplified by SNHG17, a long non-coding RNA with 109 isoforms. fRIP-seq revealed MTA1 binding near splice junctions of SNHG17 (Figure 7(b)), correlating with ES events validated by RNA-seq (Figure 7(c)). This suggests that MTA1 may either

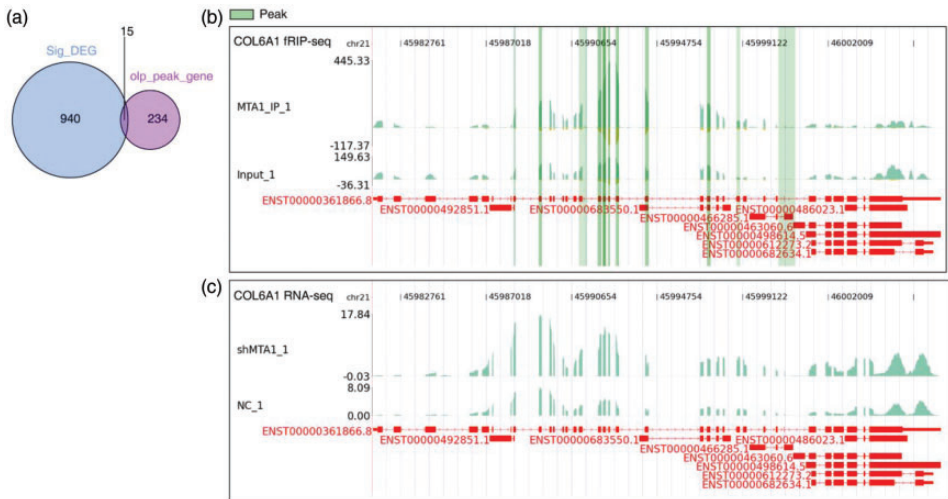


Figure 6. MTA1 may regulate RNA expression levels through integrated mechanisms. (a) Venn diagram showing overlap between DEGs and peak-associated genes; (b and c) IGV sashimi plots showing COL6A1 peak read distribution in the fRIP-seq data (b) and expression levels in the RNA-seq data (c). MTA1: metastasis-associated protein 1; DEGs: differentially expressed genes; IGV: Integrative Genomics Viewer; fRIP-seq: formaldehyde RNA immunoprecipitation sequencing; RNA-seq: RNA sequencing; COL6A1: collagen type VI alpha 1 chain.

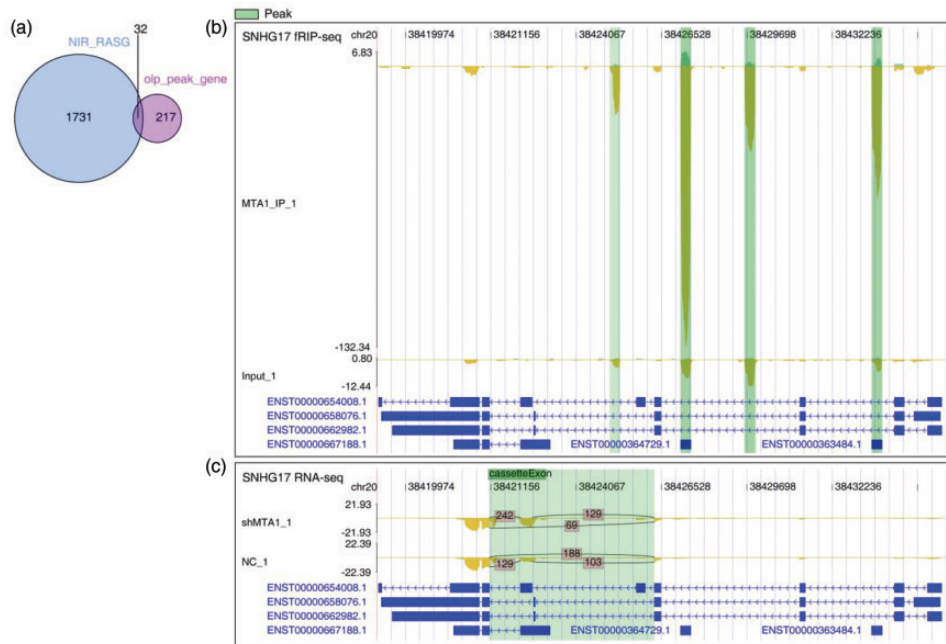


Figure 7. MTA1 may regulate RNA alternative splicing through integrated mechanisms. (a) Venn diagram showing overlap between RASGs and peak-associated genes; (b and c) IGV sashimi plots showing SNHG17 peak read distribution in the fRIP-seq data (b) and alternative splicing levels in the RNA-seq data (c). Only four transcripts are shown in the figure, although SNHG17 has 109 transcripts. MTA1: metastasis-associated protein 1; RASGs: regulated alternative splicing genes; IGV: Integrative Genomics Viewer; fRIP-seq: formaldehyde RNA immunoprecipitation sequencing; RNA-seq: RNA sequencing; SNHG17: small nucleolar RNA host gene 17.

recruit splicing machinery or sterically hinder spliceosome access, thereby altering isoform ratios and diversifying transcriptomes to favor oncogenic programs.

MTA1's dual functionality—stabilizing transcripts (COL6A1) and directing splicing (SNHG17)—appears to stem from its structural recognition of GC-rich motifs (Figure 5(f)) and splice-site-proximal RNA elements. By coupling chromatin remodeling with RNA processing, MTA1 establishes an integrated oncogenic network, driving PCa through both expression-level and isoform-specific dysregulation. These findings highlight MTA1 as a central node in RNA-centric regulatory hubs, positioning its RNA-binding domains as potential

therapeutic targets for disrupting transcriptional-splicing crosstalk in advanced malignancies.

Discussion

This study establishes MTA1 as a bifunctional regulator in PCa, integrating its well-characterized chromatin-modifying functions with a newly identified role as an RBP. By integrating RNA-seq with fRIP-seq, we demonstrate that MTA1 binds directly to GC-rich RNA motifs and influences both gene expression and alternative splicing, thereby rewiring oncogenic transcriptomes. A major strength of this work lies in its multi-omics approach, which

enabled simultaneous profiling of transcriptional and splicing alterations alongside the identification of direct RNA targets. The application of fRIP-seq under crosslinking conditions provided high-resolution mapping of MTA1–RNA interactions, whereas integrative bioinformatics revealed functional convergence in key pathways such as ECM remodeling and spliceosome assembly. Experimental validation of specific targets, including COL6A1 and SNHG17, further strengthens the mechanistic insight into MTA1's post-transcriptional roles. MTA1 preferentially binds GC-rich motifs within exons and 3' UTRs—regions critically involved in RNA stability and splicing regulation. For instance, its binding to the 3' UTR of COL6A1 is likely to stabilize this transcript, thereby sustaining ECM remodeling and tumor–stroma crosstalk. Concurrently, MTA1 binding near splice junctions of transcripts such as SNHG17 correlates with ES, suggesting that it may recruit spliceosomal components or sterically block splice site recognition. Moreover, the observed overlap of 18% between splicing-regulated genes (RASGs) and DEGs underscores MTA1's ability to coordinately modulate transcript levels and isoform diversity. Notable examples include IL6 ES, which may dampen pro-inflammatory signaling, and truncated TNFRSF12A isoforms that could promote apoptosis resistance. This dual regulatory capacity positions MTA1 as a transcriptional–splicing rheostat that fine-tunes gene output in response to microenvironmental signals. Several limitations of the current study should be noted. First, the work was conducted primarily in the PC-3 cell line; further validation across additional *in vitro* and *in vivo* models, as well as clinical specimens, would strengthen the generalizability of the findings. Second, although we identified MTA1-binding motifs and candidate

RNA targets, the structural basis of MTA1–RNA recognition remains unclear. Third, the functional impact of specific splicing alterations, such as those in IL6 and TNFRSF12A, has not been experimentally validated. Future studies should employ structural biology, crosslinking IP techniques, and functional splicing reporter assays to dissect the precise mechanisms by which MTA1 regulates RNA stability and splicing. Finally, MTA1's interactions with non-coding RNAs such as SNHG17 suggest its potential involvement in lncRNA-mediated regulatory networks, which may link chromatin organization to splicing outcomes. Unraveling these mechanisms may open new therapeutic avenues for targeting the transcriptional–splicing axis in advanced PCa, including the repurposing of GLP-1-based therapies, targeting the 20S proteasome, and exploring natural compounds such as hinokitiol as prophylactic agents with immunomodulatory and anti-tumor effects.^{26–28}

Conclusion

This study repositions MTA1 as a master regulator of PCa transcriptomes, bridging chromatin dynamics with RNA-driven oncogenesis. Its dual functionality, stabilizing mRNAs critical for microenvironment adaptation and diversifying splicing isoforms to enhance plasticity, reveals a previously unrecognized layer of cancer vulnerability. By targeting MTA1's RNA interactome, it may be possible to disrupt the transcriptional–splicing axis that drives PCa progression, offering therapeutic potential that addresses both genetic and non-genetic drivers of malignancy. Future work dissecting RNA–chromatin crosstalk and validating therapeutic strategies will be pivotal for translating these findings into clinical applications.

Acknowledgments

The authors thank Dexin Yu for assistance with manuscript preparation.

Author contributions

G.Q performed most of the experiments and data analysis; B.W conducted the fRIP-seq assays and splicing analysis; S.X and E.L provided clinical samples and interpretation; L.C supervised the research and acquired funding; Dexin Yu designed and supervised the study and revised the manuscript. All authors read and approved the final manuscript.

Data availability statement

The RNA-seq and fRIP-seq data generated in this study have been deposited in the Gene Expression Omnibus (GEO) database under accession number GSE300235 and GSE300236. The data are publicly accessible at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE300236> and <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE300235>. All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of conflicting interests

The authors declare no competing interests.

Funding

This work was supported by Key Scientific Research Projects for Higher Education Institutions of Anhui Provincial Department of Education (No. 2022AH050762), The Second Affiliated Hospital of Anhui Medical University Science and Technology Rising Star Training Project (2017KA07), and the Anhui Medical University School Funding Project (2021xkj175).

ORCID iDs

Fuhao Li  <https://orcid.org/0009-0007-9796-3928>

Lei Chen  <https://orcid.org/0009-0006-3258-695X>

Supplemental material

Supplemental material for this article is available online.

References

1. Jungfleisch J and Gebauer F. RNA-binding proteins as therapeutic targets in cancer. *RNA Biol* 2025; 22: 1–8.
2. Perr J, Langen A, Almahayni K, et al. RNA-binding proteins and glycoRNAs form domains on the cell surface for cell-penetrating peptide entry. *Cell* 2025; 188: 1878–1895.e25.
3. Cui S, Peng Q, Ma Q, et al. Crosstalk between RNA-binding proteins and non-coding RNAs in tumors: molecular mechanisms, and clinical significance. *Int J Biol Sci* 2025; 21: 2991–3010.
4. Levenson AS. Metastasis-associated protein 1-mediated antitumor and anticancer activity of dietary stilbenes for prostate cancer chemoprevention and therapy. *Semin Cancer Biol* 2022; 80: 107–117.
5. Vattam C and Pakala SB. Metastasis-associated protein 1: a potential driver and regulator of the hallmarks of cancer. *J Biosci* 2022; 47: 23.
6. Wanka G, Jueckstock J, Wild CM, et al. MTA1 as negative prognostic marker in vulvar carcinoma. *J Cancer Res Clin Oncol* 2023; 149: 6191–6201.
7. Zhang J, Wang Y, Zhang J, et al. The feedback loop between MTA1 and MTA3/TRIM21 modulates stemness of breast cancer in response to estrogen. *Cell Death Dis* 2024; 15: 597.
8. Raychaudhuri R, Lin DW and Montgomery RB. Prostate cancer: a review. *JAMA* 2025; 333: 1433–1446.
9. Ma J, Li C, Qian H, et al. MTA1: a vital modulator in prostate cancer. *Curr Protein Pept Sci* 2022; 23: 456–464.
10. Dee EC, Ng K, Shamash J, et al. Prostate cancer survivorship and global health-related quality of life. *JCO Oncol Pract* 2024; 20: 879–882.
11. Me LL. Understanding RNA-binding proteins. *Semin Cancer Biol* 2022; 86: 135–136.
12. Sciarrillo R, Wojtuszkiewicz A, Assaraf YG, et al. The role of alternative splicing in

- cancer: from oncogenesis to drug resistance. *Drug Resist Updat* 2020; 53: 100728.
13. Tsintari V, Walter B, Fend F, et al. Alternative splicing of apoptosis stimulating protein of TP53-2 (ASPP2) results in an oncogenic isoform promoting migration and therapy resistance in soft tissue sarcoma (STS). *BMC Cancer* 2022; 22: 725.
 14. Fuentes-Fayos AC, Pérez-Gómez JM, G-García ME, et al. SF3B1 inhibition disrupts malignancy and prolongs survival in glioblastoma patients through BCL2L1 splicing and mTOR/β-catenin pathways imbalances. *J Exp Clin Cancer Res* 2022; 41: 39.
 15. Maltseva D and Tonevitsky A. RNA-binding proteins regulating the CD44 alternative splicing. *Front Mol Biosci* 2023; 10: 1326148.
 16. Bigot J, Lalanne AI, Lucibello F, et al. Splicing patterns in SF3B1-mutated uveal melanoma generate shared immunogenic tumor-specific neoepitopes. *Cancer Discov* 2021; 11: 1938–1951.
 17. Venkatasubramanian M, Schwartz L, Ramachandra N, et al. Broad de-regulated U2AF1 splicing is prognostic and augments leukemic transformation via protein arginine methyltransferase activation. Epub ahead of print 8 February 2024. DOI: 10.1101/2024.02.04.578798.
 18. Holmes ME and Hertel KJ. Interdependent regulation of alternative splicing by SR and hnRNP proteins. Epub ahead of print 20 August 2024. DOI: 10.1101/2024.08.19.608666.
 19. Hamdy NM, Basalious EB, El-Sisi MG, et al. Advancements in current one-size-fits-all therapies compared to future treatment innovations for better improved chemotherapeutic outcomes: a step-toward personalized medicine. *Curr Med Res Opin* 2024; 40: 1943–1961.
 20. Sahin I, George A and Seyhan AA. Therapeutic targeting of alternative RNA splicing in gastrointestinal malignancies and other cancers. *Int J Mol Sci* 2021; 22: 11790.
 21. Russo P, Bizzarri FP, Filomena GB, et al. Relationship between loss of Y chromosome and urologic cancers: new future perspectives. *Cancers (Basel)* 2024; 16: 3766.
 22. Kim D, Langmead B and Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. *Nat Methods* 2015; 12: 357–360.
 23. Trapnell C, Williams BA, Pertea G, et al. Transcript assembly and quantification by RNA-seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat Biotechnol* 2010; 28: 511–515.
 24. Love MI, Huber W and Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014; 15: 550.
 25. Xie C, Mao X, Huang J, et al. KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. *Nucleic Acids Res* 2011; 39: W316–W322.
 26. Mostafa AM, Hamdy NM, Abdel-Rahman SZ, et al. Effect of vildagliptin and pravastatin combination on cholesterol efflux in adipocytes. *Iubmb Life* 2016; 68: 535–543.
 27. Atta H, Alzahaby N, Hamdy NM, et al. New trends in synthetic drugs and natural products targeting 20S proteasomes in cancers. *Bioorg Chem* 2023; 133: 106427.
 28. Chiang Y-F, Huang KC, Chen HY, et al. Hinokitiol inhibits breast cancer cells in vitro stemness-progression and self-renewal with apoptosis and autophagy modulation via the CD44/Nanog/SOX2/Oct4 pathway. *Int J Mol Sci* 2024; 25: 3904.