ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25sep352

Establishing AMACR as an AR-Derived Biomarker in Prostate Cancer: Metabolic and Androgen-Mediated Mechanisms

Madhab Mondal[1]; Prerna Rai[2]; Akash Baglari[3]; Divyadarshi Rai[4]; Pratiksha Chhetri[5]; Purandar Sarkar[6*]

[1,2,3,4,5] PG Department, Zoology, Darjeeling Government College, Darjeeling, West Bengal, India

Corresponding Author: Purandar Sarkar[6*]

Publication Date: 2025/09/12

Abstract: Prostate cancer (PCa) progression is primarily driven by androgen receptor (AR) signaling, which orchestrates tumor proliferation and metabolic reprogramming. Alpha-Methyl Acyl-CoA Racemase (AMACR) is a key enzyme in branched-chain fatty acid β-oxidation which is widely recognized as a diagnostic marker for PCa but its regulatory association with AR and functional implications remain unclear. Using globally available multi-omics datasets, we analyzed RNA-seq profiles from TCGA-PRAD which revealed significant overexpression of AMACR in tumors compared to normal tissues ($p < 0.001$). Elevated AMACR levels correlated with advanced Gleason scores, nodal positivity, and poor overall survival ($p = 0.027$). Functional insights from androgen stimulation and antagonism datasets, along with Chipset analyses, demonstrated direct AR occupancy at enhasssncer regions near AMACR which further confirms androgen-dependent transcriptional regulation. Protein-level evidence from immunohistochemistry and immunofluorescence supported these findings. Collectively, these results establish AMACR as an AR-regulated biomarker in PCa, implicating it in metabolic dysregulation and disease aggressiveness, and highlight its potential as a therapeutic target within the androgen-driven oncogenic network.

Keywords: *Prostate cancer, AMACR, Androgen receptor, Biomarker*.

How to Cite: Madhab Mondal; Prerna Rai; Akash Baglari; Divyadarshi Rai; Pratiksha Chhetri; Purandar Sarkar, (2025) Establishing AMACR as an AR-Derived Biomarker in Prostate Cancer: Metabolic and Androgen-Mediated Mechanisms. *International Journal of Innovative Science and Research Technology*, 10(9), 374-379. https://doi.org/10.38124/ijisrt/25sep352

I. INTRODUCTION

Prostate cancer (PCa) is among the most abundant cancers in men and also a leading cause of death [1]. The growth of PCa is largely driven by androgen receptor (AR) signaling [2]. Although androgen deprivation therapy (ADT) is one of the major procedures to fight against PCa, most of the advanced PCa cases progress to castration-resistant prostate cancer (CRPC), proving the need to identify AR-regulated factors behind the PCa treatment resistance [3]. Alpha-methylacyl-CoA racemase (AMACR) is an enzyme involved in branched-chain fatty acid metabolism. It is also a well-known diagnostic marker due to its strong overexpression in PCa [4]. However, its functional role, regulation by AR, and contribution to tumor progression remain unclear. As the central role of the AMACR is to do lipid metabolism reprogramming [5], it may serve as a metabolic link within AR signaling, influencing energy production, biosynthesis, and therapy adaptation. In this study, we integrated transcriptomic, epigenomic, and proteomic datasets, including TCGA-PRAD RNA-seq and methylation profiles, GEO datasets of androgen stimulation/inhibition, and AR ChIP-seq data. Protein-level validation was performed using immunohistochemistry (IHC) and immunofluorescence (IF) online repositories. Our results show that AMACR is highly overexpressed in PCa which correlates with aggressive features and poor survival, and it is also directly regulated by AR binding at its enhancer. These findings position AMACR as an AR-controlled biomarker linked to metabolic reprogramming in prostate cancer progression.

II. MATERIALS AND METHODS

- *Data Sources and Preprocessing*

RNA-seq and DNA methylation data were collected from the TCGA-PRAD dataset using the Genomic Data Commons (GDC) portal [6]. Transcript levels were measured in transcripts per million (TPM) and then log2-transformed for statistical analysis. DNA methylation beta values were obtained from Illumina HumanMethylation450 arrays submitted in UALCAN database [7]. Clinical information—

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25sep352

such as patient age, Gleason score, lymph node status, and survival outcomes—was matched with the molecular data for integrated analysis. All of these information also have been taken from TCGA-UALCAN database.

➢ *Differential Expression Analysis*

Differences in expression between tumor samples (n=497) and normal prostate tissue (n=52) were evaluated using unpaired Student's t-tests. Z-score normalization was applied to rank AMACR among the most overexpressed genes in prostate cancer. Variations in expression across clinical subgroups—such as age groups, Gleason scores, and lymph node status—were analyzed using one-way ANOVA followed by Tukey's post hoc test [8].

➢ *Survival Analysis*

Overall survival (OS) was assessed by dividing patients into high- and low-AMACR expression groups using the median TPM value as the cutoff. Kaplan–Meier survival curves were generated, and differences between the groups were evaluated using the log-rank test [9].

➢ *Androgen Stimulation and Antagonism Data*

GEO datasets (GSE71797 and GSE95413) were used to examine how AMACR is regulated by AR signaling. These datasets included AR-positive prostate cancer cell lines treated with either the synthetic androgen R1881 or the AR antagonist bicalutamide [10]. Differential expression analysis was conducted using GEO2R, and the results were displayed as volcano plots.

➢ *ChIP-seq Analysis*

Publicly available AR and H3K27ac ChIP-seq datasets for normal prostate tissue, localized prostate cancer, and CRPC were obtained from GEO. Peaks were mapped to the AMACR genomic region using the Integrative Genomics Viewer (IGV) to evaluate AR binding at potential enhancer sites [11].

➢ *Protein Expression Analysis*

Immunohistochemistry (IHC) images were sourced from the Human Protein Atlas to assess AMACR and AR expression patterns in benign, low-grade, and high-grade prostate cancer tissues [12]. Immunofluorescence (IF) data from published cell line studies were also examined to determine subcellular localization.

➢ *Statistical Analysis*

All statistical analyses were carried out using GraphPad Prism v8.4.2, with a p-value of less than 0.05 considered statistically significant.

III. RESULTS

➢ *AMACR is Among the Most Overexpressed Genes in Prostate Cancer*

Analysis of TCGA-PRAD RNA-seq data revealed that AMACR is significantly overexpressed in 11 different cancers (Red colour) among 33 TCGA cancers (Figure 1A). AMACR is also among the top 10 most overexpressed genes in prostate tumors compared with normal tissue (Figure 1B). Median AMACR expression in tumors was approximately 400 TPM, significantly higher than the ~10 TPM observed in normal prostate samples ($p < 0.001$) (Figure 1C).

➢ *Association OF AMACR with Clinical Parameters*

AMACR expression showed there is hardly any difference among 41-60-year age group with 61-80-year age group proves that AMACR has a steady expression in all PCa patients regardiless of ages (Figure 1E). Lymph node metastasis (Figure 1F) and Gleason score (Figure 1G) suggests that there is no difference of AMACR expression between higher metastasis group and higher cancer grade proving a steady level of AMACR expression in every stage and form of PCa. Though the methylation level has significant differences present between normal and cancer group, the beta value for both group is so small (Figure 1D) that the effect of methylation on AMACR should be taken as null or negligible.

➢ *Prognostic Implications of AMACR Expression*

Survival analysis showed that patients with high AMACR expression had significantly shorter overall survival compared to those with low expression (log-rank $p = 0.027$; Figure 1F). These results support the potential of AMACR as a prognostic marker in prostate cancer.

➢ *AMACR and AR Share Cytoplasmic Localization and Show Context-Dependent Co-Expression*

Immunofluorescence (IF) (Figure 2D) and Immunohistochemistry (IHC) (Figure 2A, 2E) analysis revealed that both AMACR and AR are mainly localized in the cytoplasm, as shown in the schematic diagram also (Figure 2C, 2F). In normal prostate tissue, AMACR expression was low, but its cytoplasmic staining intensity increased significantly in prostate cancer samples (Figure 2A, B). In contrast, AR—which is normally present in healthy prostate epithelium—showed relatively stable cytoplasmic levels between normal and tumor tissues (Figures 2E). Since both proteins share cytoplasmic localization, we assessed their relationship and found a modest positive correlation in patient samples (Figure 2G). Extending this analysis to prostate cancer cell lines, we observed that AR-high lines, such as VCaP, tended to have higher AMACR expression, whereas AR-low lines, like PC3, showed reduced AMACR levels (Figure 2H). This trend supports a context-dependent association between AR and AMACR expression in prostate cancer.

➢ *Androgen Signaling Modulates AMACR Expression, Confirmed by Multi-Dataset Transcriptomic Evidence*

Exposing the AR-positive LNCaP prostate cancer cells with synthetic androgen R1881 caused a clear and statistically significant increase in AMACR expression (Figures 3A–C). In contrast, if the cells were treated with the AR antagonist bicalutamide, it led to a marked reduction in its levels (Figures 3D–F). These androgen-driven changes were confirmed using independent RNA-seq datasets (GSE71797 and GSE95413). In both datasets, AMACR was among the most significantly upregulated genes in response to androgen treatment. In volcano plot it also appeared prominently as one of the top differentially expressed targets (Figures 3A, 3D). Consistent expression changes across treatment groups were observed in heatmaps, and statistical validation through dot plots also confirmed the significance of these trends (Figures 3C, 3F). Together, these in vitro experiments and publicly available

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25sep352

transcriptomic data provide strong and reproducible evidence that AMACR is transcriptionally regulated by AR signaling.

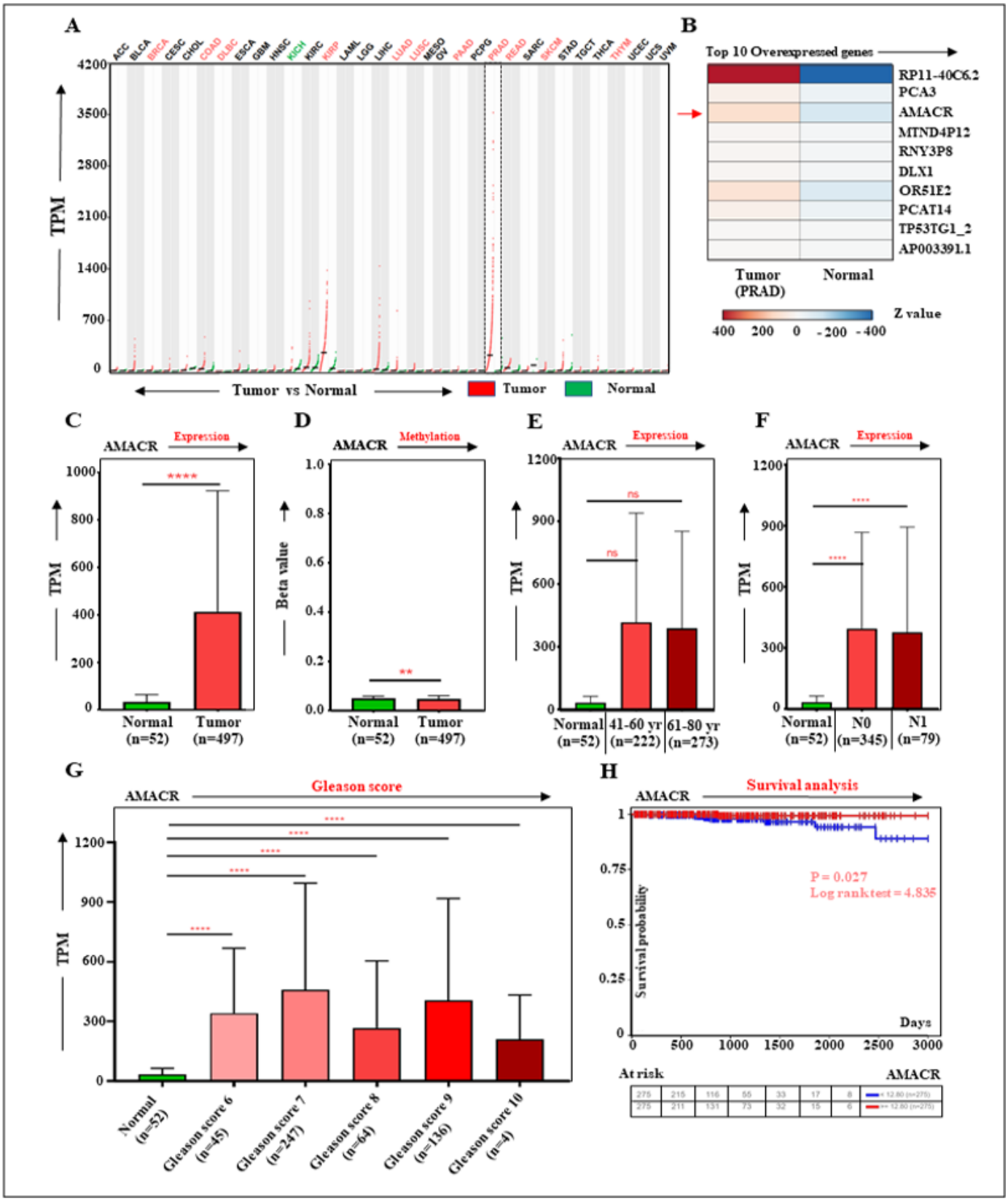

Fig 1 AMACR expression in prostate cancer and clinical correlation. (A) Pan-cancer analysis of TCGA datasets showing AMACR expression across 33 cancer types, highlighting significant overexpression in prostate cancer (PCa). (B) AMACR ranked among the top 10 most overexpressed genes in PCa compared to normal prostate tissue. (C) Box plot of AMACR transcript levels (TPM) in PCa (n=497) versus normal prostate (n=52), showing a ~40-fold increase (***p < 0.001). (D) DNA methylation beta values for AMACR locus in normal versus PCa samples, showing statistical difference but negligible effect size. (E–G) Association of AMACR expression with clinical parameters: age groups, lymph node status, and Gleason score, indicating stable

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25sep352

expression across subgroups. (H) Kaplan–Meier analysis showing shorter overall survival in patients with high AMACR expression (log-rank p = 0.027).

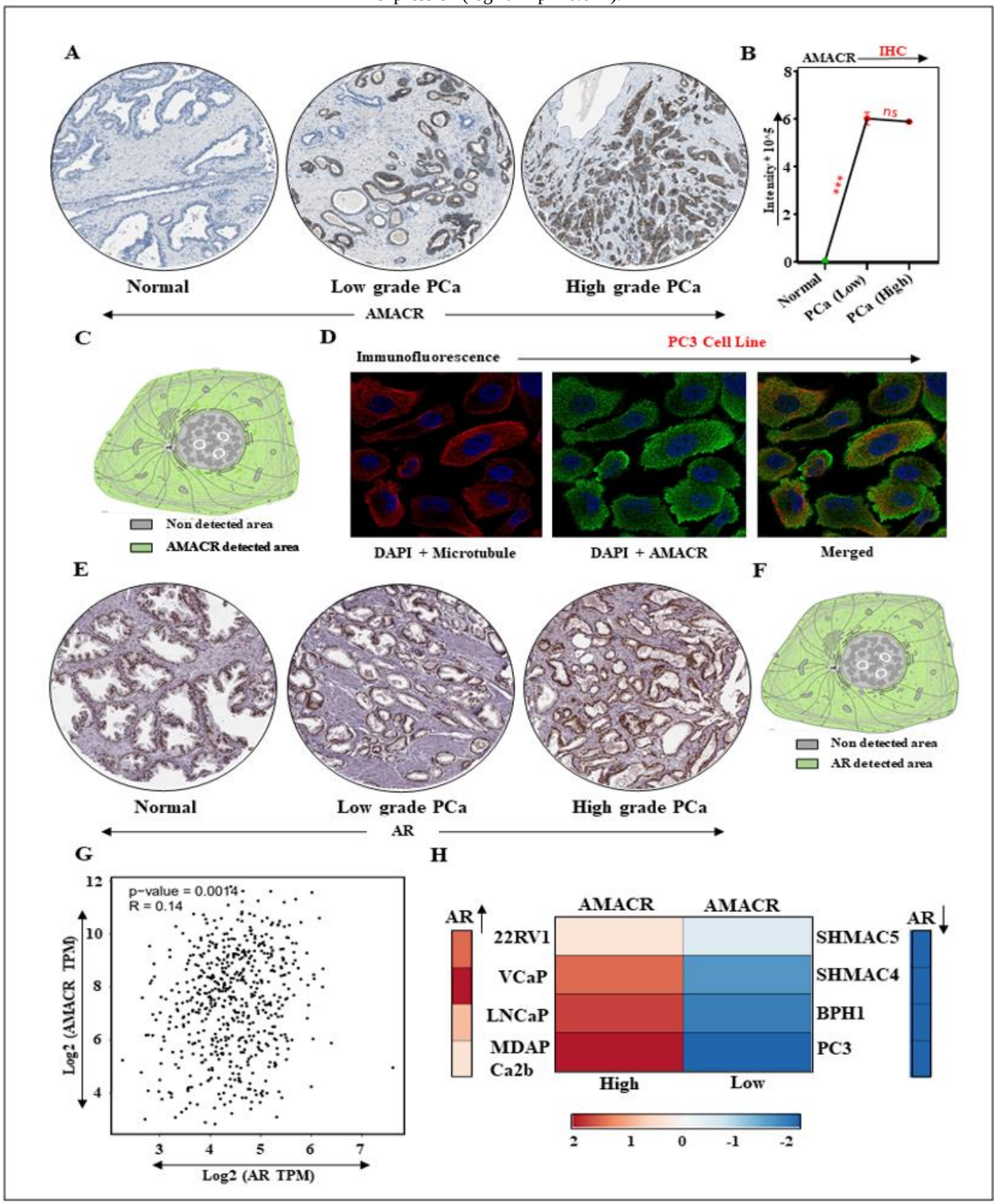

Fig 2 Protein localization and AR–AMACR association in PCa. (A–B) Representative immunohistochemistry (IHC) staining of AMACR in normal prostate, low-grade PCa, and high-grade PCa, demonstrating progressive cytoplasmic upregulation. (C) Schematic model of cytoplasmic localization of AMACR and AR. (D) Immunofluorescence (IF) analysis showing cytoplasmic distribution of AMACR and AR in prostate cancer cells. (E–F) IHC images of AR in normal versus tumor tissues, showing relatively stable cytoplasmic levels. (G) Correlation plot of AMACR and AR expression in TCGA patient samples, showing a

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25sep352

modest positive relationship. (H) Comparative expression across prostate cancer cell lines, with AR-high lines (VCaP) showing higher AMACR expression than AR-low lines (PC3).

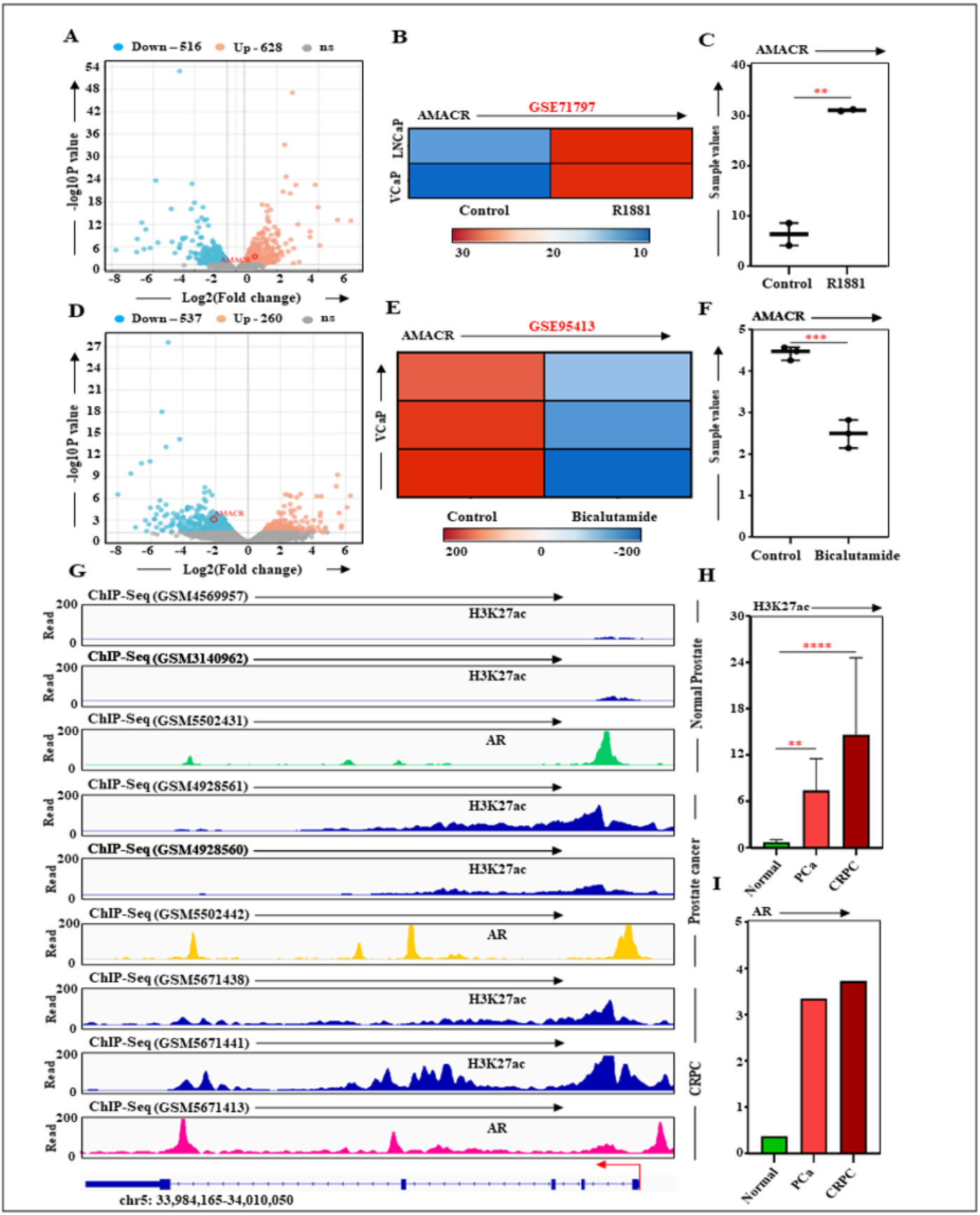

Fig 3 Androgen regulation and epigenomic activation of AMACR. (A–C) Effect of androgen stimulation (R1881) in AR-positive LNCaP cells: volcano plot, heatmap, and dot plots confirm AMACR as a significantly upregulated androgen-responsive gene. (D–F) AR antagonism (bicalutamide) reduces AMACR expression in LNCaP and VCaP cells; results validated in GEO datasets

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25sep352

(GSE71797 and GSE95413). (G) AR ChIP-seq tracks showing binding at enhancer regions near AMACR in normal prostate and PCa. (H–I) H3K27ac ChIP-seq enrichment across disease progression (Normal → PCa → CRPC), with bar graph quantifying enhancer activation (****p < 0.0001).

➢ *H3K27ac-Mediated Enhancer Activation Strengthens AR-Driven AMACR Regulation in Prostate Cancer*

ChIP-seq analysis revealed detectable AR binding at enhancer regions near the AMACR gene in both normal prostate tissue and prostate cancer samples, which aligns with AR's normal physiological function in prostate cells (Figure 3G). Notably, the activation-related histone mark H3K27ac was almost negligible in normal prostate while significantly higher in prostate cancer samples and rose even further in castration-resistant prostate cancer (CRPC). This increase in H3K27ac suggests that these enhancers are in an epigenetically activated state, potentially boosting AR-driven transcription of AMACR as the disease advances. The bar graph of ChIP-seq peak intensities supports this trend, showing a stepwise rise in enhancer activity from normal tissue to localized cancer, and finally to CRPC (Figure 3H, 3I). Together, these results indicate that while AR binding to the AMACR locus is a standard feature of prostate cells, cancer-related enhancer activation via H3K27ac enrichment magnifies AR's influence, leading to increased AMACR expression in more advanced stages of the disease.

IV. DISCUSSION

This study establishes a mechanistic link between androgen receptor (AR) signaling and transcriptional regulation of AMACR in PCa. Using transcriptomic, proteomic, and epigenomic datasets, we showed that AMACR which is already a diagnostic marker, is significantly overexpressed in PCa and correlates with aggressive disease and poor survival. Protein-level analysis revealed cytoplasmic localization of both AR and AMACR where AMACR upregulated in cancer tissues but AR levels remained stable. In patient samples, a modest correlation was found where AR-high cell lines such as VCaP expressed high AMACR, whereas AR-low lines had reduced levels. Functionally, androgen stimulation (R1881) increased AMACR expression, while AR blockade (bicalutamide) reduced it, confirming androgen responsiveness. ChIP-seq analyses revealed AR binding at AMACR enhancer regions in both normal and malignant tissue, but with marked H3K27ac enrichment in PCa and CRPC was causing an enhancer activation which indeed amplified AR-driven AMACR transcription during the cancer progression. This suggests a model where baseline enhancer binding in normal prostate becomes hyperactivated in cancer causing an elevated AMACR and fueling metabolic reprogramming through branched-chain fatty acid β-oxidation to support biosynthesis, energy production, and therapy resistance. These findings position AMACR as more than a biomarker proving its identification as an AR-driven metabolic effector in PCa biology. Targeting AMACR alongside AR-directed therapies may provide new therapeutic opportunities. While limited by reliance on public datasets, this multi-omics analysis highlights AMACR as a promising functional target warranting in vivo validation.

➢ *Author Contributions*

MM, PR, AB, DR, PC and PSR together wrote the paper. MM and PSR conceived the idea and finally edited the manuscript.

➢ *Conflict of Interest Statement*

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ACKNOWLEDGMENTS

The authors apologize to colleagues whose research could not be cited due to space constraint. The authors thank Dr. Prajjal Chandra Lama, Officer-in-charge, Darjeeling Government College, Darjeeling, for his esteemed support. Authors also thank Dr. Rujas Yonle, HoD, Department of Zoology, Darjeeling Government College, Darjeeling for their valuable suggestions. Authors also thank all the colleagues of Department of Zoology, Darjeeling Government College, Darjeeling, for their help and support.

REFERENCES

[1]. Rawla P. Epidemiology of Prostate Cancer. World J Oncol 2019; 10: 63–89.
[2]. Aurilio G, Cimadamore A, Mazzucchelli R, et al. Androgen Receptor Signaling Pathway in Prostate Cancer: From Genetics to Clinical Applications. Cells 2020; 9: 2653.
[3]. Huang Y, Jiang X, Liang X, et al. Molecular and cellular mechanisms of castration resistant prostate cancer. Oncol Lett 2018; 15: 6063–6076.
[4]. Li W, Cagle PT, Botero RC, et al. Significance of overexpression of alpha methylacyl-coenzyme A racemase in hepatocellular carcinoma. J Exp Clin Cancer Res CR 2008; 27: 2.
[5]. Lloyd MD, Yevglevskis M, Lee GL, et al. α-Methylacyl-CoA racemase (AMACR): Metabolic enzyme, drug metabolizer and cancer marker P504S. Prog Lipid Res 2013; 52: 220–230.
[6]. Jensen MA, Ferretti V, Grossman RL, et al. The NCI Genomic Data Commons as an engine for precision medicine. Blood 2017; 130: 453–459.
[7]. Chandrashekar DS, Karthikeyan SK, Korla PK, et al. UALCAN: An update to the integrated cancer data analysis platform. Neoplasia 2022; 25: 18–27.
[8]. McHugh ML. Multiple comparison analysis testing in ANOVA. Biochem Medica 2011; 21: 203–209.
[9]. Goel MK, Khanna P, Kishore J. Understanding survival analysis: Kaplan-Meier estimate. Int J Ayurveda Res 2010; 1: 274–278.
[10]. Wang T, Alavian MR, Goel HL, et al. Bicalutamide inhibits androgen-mediated adhesion of prostate cancer cells exposed to ionizing radiation. The Prostate 2008; 68: 1734–1742.
[11]. Robinson JT, Thorvaldsdóttir H, Winckler W, et al. Integrative Genomics Viewer. Nat Biotechnol 2011; 29: 24–26.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25sep352

[12]. Thul PJ, Lindskog C. The human protein atlas: A spatial map of the human proteome. Protein Sci Publ Protein Soc 2018; 27: 233–244.