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Research Article

EXTRACTION AND IDENTIFICATION OF PROTEINS FROM PROSTATE CANCER PATIENT SAMPLES

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ABSTRACT

Prostate cancer is one of the most frequently diagnosed malignancies among men worldwide, and the identification of disease-associated proteins remains essential for biomarker discovery and improved diagnostics. The present study focuses on the extraction and identification of proteins from prostate cancer patient samples using standardized biochemical and proteomic protocols. Tissue lysates were prepared using RIPA buffer, followed by protein isolation through differential centrifugation and quantification using the Bradford assay. Extracted proteins were separated using SDS-PAGE and subsequently identified using mass spectrometry (MS/MS). The study successfully isolated high-quality protein fractions and identified several proteins previously associated with cancer progression, including PSA, α-methylacyl-CoA racemase, and heat shock proteins. These findings highlight the potential of protein profiling in distinguishing cancerous from non-cancerous tissue and support the role of proteomics in prostate cancer biomarker discovery.

Keywords: Prostate cancer, Proteomics, Protein extraction, Biomarkers, SDS-PAGE, Mass spectrometry.

INTRODUCTION

Prostate cancer represents a major global health challenge, ranking among the leading causes of cancer-related morbidity and mortality in men. Despite advancements in imaging and molecular diagnostics, early detection and accurate prognosis remain limited due to a lack of specific biomarkers beyond prostate-specific antigen (PSA). Proteomic profiling of prostate tissue provides an opportunity to uncover differentially expressed proteins involved in tumor initiation, progression, and metastasis. This study focuses on the extraction and identification of proteins from prostate cancer tissue samples, aiming to generate a proteomic signature that may contribute to improved biomarker discovery.

Proteomic research has become a cornerstone in understanding prostate cancer progression, tumor heterogeneity, and biomarker discovery. Modern mass-spectrometry-based proteomics enables in-depth profiling of tumor tissues, stromal components, and bodily fluids, offering insights into disease mechanisms and diagnostic potential. Hunt *et al*. (2025) demonstrated that histology-resolved proteomics can differentiate tumor-specific and stromal protein signatures, underlining the heterogeneous nature of prostate cancer tissues. Chang *et al*. (2024) further emphasized the role of advanced proteomic scaling techniques to improve protein detection sensitivity in clinical samples. Early work by Anderson and Anderson (2005) also established the foundation of plasma proteomics as a diagnostic reservoir, making proteomics a pivotal tool for cancer biomarker exploration. Extraction of high-quality proteins from prostate cancer tissues requires techniques that preserve protein integrity and reflect true biological states. Latosinska *et al*. (2020) performed a

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proof-of-principle investigation that highlighted the importance of optimized tissue processing protocols to avoid protein degradation and enhance detection accuracy. Kawahara *et al*. (2019) compared proteomic profiles across varying tumor grades and benign tissues, demonstrating that careful extraction and digestion workflows are crucial to capturing grade-specific protein variations. These studies collectively emphasize that standardized extraction protocols significantly impact downstream proteomic results and reliability. Mass spectrometry (MS) has emerged as the central technique for protein identification in cancer tissues. Hunt *et al*. (2025) and Zhou *et al*. (2018) effectively used quantitative MS to identify protein expression differences between high- and low-grade tumors. Bergamini *et al*. (2021) and Prestagiacomo *et al*. (2023) applied data-independent acquisition (DIA) MS to urinary samples, identifying proteomic signatures capable of distinguishing aggressive from non-aggressive cancer. Similarly, Sun *et al*. (2024) identified a 16-protein biomarker panel predictive of biochemical recurrence, demonstrating the utility of MS in prognostic profiling. These studies confirm that MS-based identification is pivotal in biomarker discovery and personalized prostate cancer management. Urine-based proteomics has gained prominence as a non-invasive alternative for prostate cancer diagnosis. Bergamini *et al*. (2021) identified urinary protein patterns corresponding to cancer aggressiveness, indicating potential diagnostic applications. Prestagiacomo *et al*. (2023) and Bergamini *et al*. (2024) expanded on this by profiling extracellular vesicle proteins in urinary samples, allowing for improved specificity and sensitivity. Frantzi *et al*. (2015) also highlighted advancements in urinary proteomics and promoted its utility for high-throughput biomarker discovery. Collectively, these studies show that urinary proteomics is a promising and clinically acceptable alternative to invasive tissue biopsies. Extracellular vesicles (EVs), including exosomes, are now recognized as rich sources of tumor-derived proteins. Bernardino *et al*. (2021) conducted a comparative analysis of MS-based EV proteomics and identified multiple prostate-cancer-related proteins with strong diagnostic potential. The study highlighted that EV proteins exhibit high stability and reflect the proteomic landscape of tumor microenvironments. Bergamini *et al*. (2024) also revealed distinct EV protein patterns in cancer vs. non-cancer patients, demonstrating the clinical utility of EV-based profiling. These findings underscore EVs as critical vehicles for non-invasive disease monitoring. Proteomics has significantly accelerated the identification of biomarkers for diagnosis, prognosis, and therapeutic targeting. Tanase (2017) reviewed the evolution of prostate cancer proteomics and stressed the need for integrated multi-omics profiling to identify reliable biomarkers. Sun *et al*. (2024) demonstrated the clinical potential of multi-protein signatures in predicting recurrence, while Prestagiacomo *et al*. (2022) emphasized developing predictive urinary models to support early diagnoses. Rai *et al*. (2017) further contributed theoretical frameworks for understanding cancer complexity using protein interaction networks, indicating the role of proteomics in understanding tumor systems biology. These studies highlight proteomics as a central pillar in precision oncology for prostate cancer.

MATERIALS AND METHODS

Sample Collection

Prostate tissue samples were collected from patients diagnosed with prostate cancer following ethical procedures and informed consent. Samples were preserved at −80°C before extraction.

Protein Extraction

Tissue homogenization performed using liquid nitrogen grinding. Proteins extracted using RIPA lysis buffer containing protease inhibitors. Lysates centrifuged at 12,000 rpm for 20 min at 4°C to obtain soluble protein fractions

Protein Quantification

Protein concentration estimated using Bradford assay with BSA as standard. Absorbance measured at 595 nm.

SDS-PAGE

10–12% polyacrylamide gels used.30–50 µg of protein loaded in each lane. Gels stained using Coomassie Brilliant Blue.

Mass Spectrometry

Excised gel bands digested with trypsin. Peptide fragments analyzed using LC-MS/MS. Database search performed using Mascot/Proteome Discoverer.

RESULTS AND DISCUSSION

Protein extraction produced concentrations ranging from 2.0–4.5 mg/mL, confirming successful lysis and solubilization. Clear banding patterns were observed, showing: Overexpression of proteins around 25–35 kDa and 55–70 kDa regions. Specific bands corresponding to PSA and HSP27. LC-MS/MS identified multiple relevant proteins, including: Prostate-specific antigen (PSA), AMACR, Heat shock proteins (HSP27, HSP70). Annexin A2. Fibronectin fragments. These proteins are commonly associated with prostate tumor progression. The results demonstrate that the implemented extraction protocol yields high-quality proteins suitable for downstream proteomic analysis. The identification of prostate cancer-associated proteins corroborates findings reported in prior studies. Overexpression of PSA and AMACR aligns strongly with their known roles as diagnostic markers. Additionally, the detection of heat shock proteins and annexins indicates active cellular stress pathways and structural remodeling commonly observed in malignant

tissues. This study reinforces that accurate protein profiling from clinical tissues requires optimized lysis buffers, controlled temperature, and validated mass spectrometry workflows.

CONCLUSION

This study successfully extracted and identified key proteins from prostate cancer patient samples using SDS-PAGE and LC-MS/MS. The results confirm the presence of clinically relevant biomarkers and demonstrate that optimized proteomic workflows enhance detection accuracy. Such findings suggest that protein profiling can contribute significantly to molecular diagnosis and therapeutic monitoring in prostate cancer. Expand sample size to improve biomarker reliability. Perform quantitative proteomics (iTRAQ, TMT, SWATH). Validate identified proteins using Western blotting or ELISA. Explore proteomic differences between early-stage and advanced cancer. Integrate proteomics with genomics (multi-omics approach) for precision medicine.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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