Applications of Prostate Cancer Proteomics: A Review

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Prostate cancer is the second most prevalent cancer in men and one of the leading causes of mortality globally. Therefore, clinical prostate cancer therapy requires better prognosis and treatment methods. Research on proteomics has enhanced the understanding of the processes underlying tumorigenesis, cancer cell migration, and metastasis. Considering that proteins are the drivers of most cellular responses and the targets for drug delivery, a methodical analysis of the proteome alterations taking place during the initiation and development of prostate cancer might lead to scientific breakthroughs. This review is a systematic literature search to retrieve primary research articles related to prostate cancer proteomics disease mechanisms and aims to discuss and compare current proteomics methods in both the clinical and research context. Aberrant regulation of lipid metabolism due to abnormal expression of the fatty acid synthase (FASN) enzyme has been extensively documented in available literature. An abnormal increase in the expression of this enzyme was associated with prostate cancer. Lastly, prostate cancer diagnosis and prognosis has been enhanced through the use of biomarkers. The use of prostate cancer biomarkers has also been extended to biofluid analysis of extracellular vesicles. Furthermore, profiling of total proteins in prostate cancer cells will help the drug development process to optimise drug activity while limiting cytotoxicity to non-target cells.

INTRODUCTION

Prostate cancer (PCa) is one of the most common men's cancers worldwide. According to the Office for National Statistics (2019) cancer registration report, PCa was the most common cancer in men in the UK with over 40,000 cases diagnosed in 2017 alone. The current methods of determining the correct therapy for a patient diagnosed with PCa depend almost entirely on Prostate-Specific Antigen (PSA) levels, histopathological features, diagnostic imaging, and clinical assessment of the severity of the disease (Jadvar et al., 2020). Recent developments in genomic technology have changed our perception of complex molecular, genetic, metabolomic, epigenetic, and transcriptomic remodelling in PCa. Extensive genomic and transcriptomic analysis has been used to investigate driver mutations and expressional

changes in prostate cancer (Felici et al., 2012; Wyatt et al., 2014; Iglesias-Gato et al., 2016; Yuan et al., 2016). Nevertheless, the discoveries into the genetic environment of PCa are yet to have a major effect on the clinical care of PCa patients. Furthermore, these methods cannot detect all molecular alterations that affect the tumour outcome (Ylipaa et al., 2015). Methodologies for proteome-wide quantitative profiling have the ability to reveal previously identified molecular linkages between genotype and phenotype and to promote the discovery of reliable prognostic biomarkers or effective drug targets for enhanced PCa treatment (Mann et al., 2013; Iglesias-Gato et al., 2016).

Understanding molecular events in cancer requires thorough investigation of the proteome (Boja and Rodriguez, 2014). In recent decades, mass spectrometric methods have enabled high throughput analysis of clinical patient samples (Schubert et al., 2017). A significant development for global protein quantification was the incorporation of isobaric tags, which made it possible to measure proteins globally through several samples in a single experiment (Pappireddi et al., 2019). Classification of diseases according to their molecular characteristics has helped to manage other types of cancer (e.g., breast cancer), and it is expected that this might be feasible for PCa with the help of novel proteomics approaches. This review is the result of a systematic Google Scholar literature search to retrieve thirty primary research articles related to prostate cancer proteomics disease mechanisms. Research conducted over the past four years has been reviewed, summarised, and critically evaluated. The aim of this literature review is to compare and contrast existing proteomics approaches in both clinical and research settings.

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Table 1. Overview of the primary literature research articles published over the last four years covering prostate cancer proteomics disease mechanisms. A systematic literature search on Google Scholar database yielded thirty primary literature research papers based on prostate cancer proteomics disease mechanisms and has been summarised in Table 1. The table is divided into four main sections namely, reference (REF), proteomic approach used, sample size which is subdivided into disease and control groups, and proteins validated. Overall, the data indicates that liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the most used proteomic approach and that the proteins validated varied across the studies.

Overview of Prostate cancer Proteomics Studies (cont.)				
REF	Proteomic Approach	Sample Size		Proteins Validated
		Disease	Control	
(Asuthkar et al., 2016)	LC-MS/MS	Prostate adenocarcinoma samples (*n* = 60)	Normal prostate tissues samples (*n* = 9)	TRPM8 validated by High-throughput proteomics analysis
(Carbonetti et al., 2019)	Western blotting Co-immunoprecipitation LC/MS	Animal models Male BALB/c nude mice Cell-lines Male LNCaP and PC3 cells		FABP5, FASN, and MAGL validated by High-throughput proteomics analysis
(Dhondt et al.,2020)	LC-MS/MS	Urine samples from patients with prostate cancer (prior to and after treatment) and benign prostatic hyperplasia (BPH)		——
(Drake et al., 2016)	Tandem mass spectrometry (MS/MS)	Tissue from lethal metastatic castration-resistant prostate cancer (CRPC) patients		——
(Garrido-Rodríguez et al., 2019)	Shotgun approach by nanoLC-MS/MS SWATH proteomic approach	Human prostate cancer cell lines: LNCaP androgen-sensitive	Human prostate cancer cell lines: DU145 androgen-insensitive	Down-regulation of (NUSAP1) under DU145 confluence validated by SWATH proteomic approach
(Goh et al., 2019)	network-based proteomics approaches qPSP and PFSNet	60 samples from 3 patients: 12 (6 normal, 6 acinars) from 1 and 2 patients; 36 (12 normal, 12 acinars, 12 ductals) from 3 patients		——
(Han et al., 2018)	iTRAQ quantitative proteomic analysis High pH reverse phase (RP) fractionation NanoLC-MS/MS analysis	Human prostate cancer cell lines (PC-3, DU145) and clinical samples		Upregulation of FZD6 (tumour suppressor) validated by High-throughput proteomics analysis
(He et al., 2018)	Liquid chromatography (LC)–electrospray ionization (ESI) tandem MS (MS/MS) analysis	AIPC cell line PC3 and androgen-dependent prostate cancer (ADPC) cell line LNCaP		Seven proteins both down-regulated by miR-200b mimics and up-regulated by miR-200b inhibitor, TM4SF1, YAP1, PPP1R2, MARCKS, RTN4, GLIPR2 and SUCLG1 validated by label free proteomics
(Hornung et al., 2020)	LC-MS/MS analysis Western blotting ELONA and ELISA	Cell lines VCaP cells and LNCaP cells		YBX1 validated by LC-MS/MS analysis Western blotting ELONA and ELISA
(Iglesias-Gato et al., 2018)	Mass spectrometry–based proteomic analysis	Bone metastasis samples from men with prostate cancer cohort of prostate cancer bone metastases (*n* = 65)		——
(Ishizuya et al., 2020)	SDS-PAGE Western blotting LC-MS/MS	Total of 36 patients with metastatic prostate cancer untreated (*n* = 8), well-controlled with primary androgen deprivation therapy (ADT) (*n* = 8) and CRPC (*n* = 20)		ACTN4 validated by SDS-PAGE Western blotting LC-MS/MS
(Kim et al., 2016)	Multiplexed SRM-MS	EPS urine samples cancer patients (*n* = 90)	EPS urine samples normal controls (*n* = 48)	ANXA3, IDHC, PEDF, PRDX6, SERA and TGM4 validated by Multiplexed SRM-MS
(Latonen et al., 2018)	NanoRPLC-MSTOF MS/MS SWATH-MS	Fresh-frozen tissue specimens from 10 BPH, 17 untreated PC, & 11 CRPC samples		MDH2 and ACO2 validated by proteomic mass spectrometry analysis
(Li et al., 2017)	LTQ Orbitrap LC-MS/MS mass spectrometry	Human prostate carcinoma cell lines PC-3 and DU-145		CD44, α2 integrin, β1 integrin, CD49f, CD133, CD59, EphA2, CD138, transferrin receptor, profilin
(Nguyen et al., 2018)	UltiMate 3000 RSLC nano LC system coupled to an LTQ-Orbitrap mass spectrometer	Patient-derived explant (PDE) discovery cohort (*n* = 16)	Patient-derived explant (PDE) validation cohort (*n* = 30)	Eight proteins were altered across both cohorts by the most potent inhibitor, AUY922, including TIMP1, SERPINA3 and CYP51A

Overview of Prostate cancer Proteomics Studies (cont.)				
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(Nguyen et al., 2019)	UltiMate 3000 RSLC nano LC system coupled to an LTQ-Orbitrap mass spectrometer Western Blot	Human prostate specimens from radical prostatectomy RWPE-2 cell line		Increased expression of LOXL2 & DDR2 in CAF validated by enzymatic assays and Western blotting analyses
(Park et al., 2017)	LC-MS/MS	prostate epithelial cell line RWPE-1 prostate cancer cell line LNCaP prostate cancer cell lines Du145 and PC3		Yin Yang 1 (YY1) Validated by LC-MS/MS
(Rodríguez-Blanco et al., 2018)	Orbitrap-MS/MS Tissue microarrays (TMAs)	PCa tissue samples (n = 34)	PCa-adjacent normal control samples (n = 33)	LOX5 and AGR2 validated by Orbitrap-MS/MS
(Sequeiros et al., 2016)	Western blotting Tissue microarrays (TMAs) Orbitrap-MS/MS	107 urine samples divided into 2 groups: PCa patient samples (n = 53), which include 22 low-grade PCa & 31 high-grade PCa; and control samples (n = 54).		ADSV-TGM4 CD63-GLPK5-SPHM-PSA-PAPP validated by immunohistochemistry assays in tissue microarrays (TMAs)
(Singh and Sharma, 2020)	SWATH-LC-MS/MS approach	Prostate tumour samples (n = 492)	Normal prostate samples (n = 152)	GOT1, HNRNPA2B1, MAPK1, PAK2, UBE2N, and YWHAB validated by SWATH proteomic approach
(Sinha et al., 2019)	Shotgun Proteomics	Cohort of 76 patients diagnosed with sporadic, localized, treatment-naive intermediate-risk prostate cancer		MED12, FOXA1, NKX3-1, and PTEN
(Staunton et al., 2016)	Label-free nLC-MS/MS	Patient tissue sample tumour epithelial cells and their associated stromal cells (n = 1)	Patient tissue sample benign epithelial cells and their associated stromal cells (n = 1)	——
(Stelloo et al., 2018)	nanoLC-MS/MS on an Orbitrap Fusion Tribrid mass spectrometer	Prostate tumour samples (n = 496)	Normal prostate samples (n = 53)	ARID1a, BRG1, TLE3, PARP1, RCC1 and FOXA1 validated by co-immunoprecipitation
(Totten et al., 2018)	Multi-lectin affinity chromatography (M-LAC) Reversed-phase (RP) fractionation LC-MS/MS	Serum samples were taken from an existing serum bank collected in patients immediately before prostate cancer surgery or from men with elevated serum PSA levels, known BPH, and two or more previous negative prostate biopsies.		Glycoform-specific modifications between BPH and PCa have been detected between CD163, C4A & ATRN proteins in the PHA-L / E fraction and between C4BPB & AZGP1 glycoforms in the AAL fraction.
(Welton et al., 2016)	Electrophoresis and immunoblotting SOMAscan® array	Plasma & urine specimens from healthy donors	Plasma & urine specimens from metastatic prostate cancer patients	——
(Xie et al., 2019)	UPLC-MS analysis	22RV1 prostate cancer cell line	22RV1 cells control group	——
(Xu et al., 2016)	iTRAQ labelling High pH reversed-phase fractionation Reverse-phase nanoLC-MS/MS analysis western blot analysis	Human prostate cancer cell line PC3	PC3 cells control group	One candidate protein, HSPA1A identified by western blot analysis
(Zhang et al., 2019)	MSA-PC-3 microarray TCGA RNA-seq	Metastatic CRPC (n = 159)	Primary prostate tumours (n = 500)	GDF15 as an immediate target of MSA in prostate cancer cells
(Zhang et al., 2018)	Q Exactive Plus Orbitrap LC-MS/MS EASY-nLC 1000 system	Patient prostate cancer tissue (n = 4)	Adjacent normal prostate tissue (n = 4)	Overexpression of PDGF-B in tumour tissues
(Zhou et al., 2019)	High-pH reversed-phase liquid chromatography (LC) LC-SPS-MS3	PCa tissue samples (n = 18)	PCa-adjacent normal control samples (n = 9)	Thirteen integrin complexes were significantly downregulated in both low- and high-grade PCa, & four Prothymosin alpha (ProTα) complexes were significantly upregulated in high-grade PCa

LITERATURE SEARCH AND REVIEW STRATEGY

A systematic literature search was conducted via Google Scholar. Records were retrieved based on the following search criteria from Google Scholar database: (1) prostate cancer proteomics disease mechanisms and (2) timespan: 2016-2020. As shown in Table 1, the search resulted in the retrieval of 30 primary literature research articles.

PROSTATE CANCER PROTEOMICS DISEASE MECHANISMS

Prostate cancer (PCa) is a highly heterogeneous disorder; several patients display an aggressive form of the disease with progression and metastasis, whereas other patients display an indolent form of it, having low propensity for progression (Wang et al., 2018). Scientists have identified three stages of human prostate tumours which include: intraepithelial neoplasia, adenocarcinoma androgen-dependent, and adenocarcinoma androgen-independent or castration-resistant. Over the years, developments in molecular technology have made very rapid progress, contributing to the better understanding of the proteomics and genomics events responsible for the initial development and progression of prostate cancer. Studies have shown that the genome of prostate cancer displays a relatively low rate of mutations compared to other cancers (Fiñones et al., 2013; Testa et al., 2019). Unlike genomic and transcriptomic technologies, proteomic technologies based on mass spectrometry (MS) allow comprehensive and direct protein analysis and have therefore been extensively used in the proteomic profiling of clinical specimens (Murray et al., 2017).

Prostate cancer stems from the loss of the regulation of lipid metabolism pathways. Both synthesised and diet acquired lipids play a significant role in the development of prostate tumours. At the proteomic level, enzymes of lipid metabolism have been linked to metastasis of prostate cancer. For instance, proteomic analysis using Western Blot and co-immunoprecipitation Liquid Chromatography–Mass Spectrometry (LC/MS) have heavily implicated the enzyme fatty acid synthase (FASN) in the metastasis of prostate cancer (Carbonetti et al., 2019). Researchers have reported a remarkable upregulation of the genes responsible for FASN in prostate cancer mice. Moreover, inhibition of the enzyme limited the growth of the tumour cells. A characteristic up-regulation of frizzled class receptor 6 (FZD6) has been shown to suppress the development of prostate cancer cells, suggesting the involvement of the Wingless-related integration site (Wnt) signalling pathway in prostate cancer (Han et al., 2018). The findings suggested that suppressing Wnt signalling pathway might help to prevent disease progression.

TARGETING METABOLIC PATHWAY PROTEINS

Proteomic analysis using LC-MS/MS have revealed the involvement of the Wnt signalling pathway in the prostate. Wnt signalling through transient receptor potential melas-tatin member 8 (TRPM8) triggers the stemness of prostate cancer. The proteomic analysis of the cellular signalling mechanism revealed the participation of FZD6 in the signalling of the prostate tumour. Proliferation and migration of the tumours were shown to be inhibited by Luteolin, further confirming the participation of TRPM8 in the signalling (Asuthkar et al., 2016). A liquid chromatography analysis of the protein regulated by miR-200b confirmed the findings presented by Asuthkar et al. (2016). He et al. (2018) used prostate cancer cell lines to demonstrate the involvement of transmembrane 4 L6 family member 1 (TM4SF1), yes-associated protein 1 (YAP1), Protein phosphatase inhibitor 2 (PPP1R2), Myristoylated alanine-rich C-kinase substrate (MARCKS), and Reticulon 4 (RTN4) inhibitors in reducing the progression of prostate cancer. The inhibitors analysed targeted the Wnt pathway. Also, 13 proteins were found to be upregulated in miR-200b mimics (Park et al., 2017). These findings highlight the importance of proteomic analysis in understanding the cellular signalling of prostate cancer. The application of proteomics tools in the field of prostate cancer has opened exciting opportunities for the study of protein-inhibitor based therapeutic interventions. Currently, the majority of anti-prostate cancer drugs in clinical trials are based on cellular signalling inhibition (Xie et al., 2019).

The investigation of the metabolic pathways involved in the aetiology and progression of PCa has also provided insight into the mechanisms involved in the regulation of enzymes in the fatty acid biosynthetic pathways. For instance, LC/MS and Western Blot analysis in animal models have provided evidence of the dysregulation in the fatty acid biosynthetic pathway. FASN and monoacylglycerol lipase (MAGL) are enzymes which create pools of cellular fatty acids whereas fatty acid binding protein 5 (FABP5) is an intracellular chaperone which delivers fatty acids to nuclear receptors to augment PCa metastasis. As FABP5, FASN, and MAGL have been shown to be independently involved in PCa progression, the researchers hypothesised that FABP5 represents a central mechanism that links cytosolic lipid metabolism to pro-metastatic signalling of nuclear receptors. Examination of FABP5 function in mice has shown that the ability of MAGL and FASN to promote nuclear receptor activation and PCa metastasis depends on FABP5 expression (Figure 1) (Carbonetti et al., 2019).

BIOMARKERS FOR PROSTATE CANCER

Pathway regulation through phosphorylation has also been used to enhance the understanding of the mechanisms of prostate cancer. Transcription factors are tightly regulated through phosphorylation pathways; thus, the application of phosphoproteomic datasets to analyse pathways may provide insights into the mechanism of prostate cancer. Drake et al. (2016) demonstrated the existence of patient unique signalling networks in patients with castration-resistant prostate cancer (CRPC). The researchers reported significant differences in signalling pathways among CRPC patients.
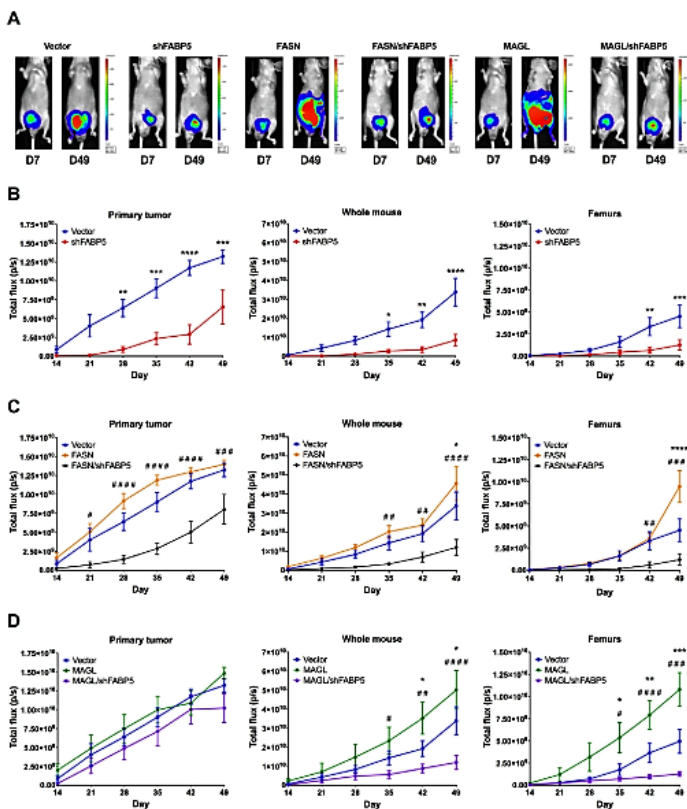
Figure 1: FABP5 is required for in vivo prostate cancer metastasis mediated by FASN and MAGL. (A) Demonstrative luciferase signals of PC3-Luc cells expressing vector, MAGL, or FASN on days 7 (D7) and 49 (D49) respectively. Signals were seen for PC3-Luc cells that express FABP5 shRNA (shFABP5) as well as those expressing FABP5 shRNA while overexpressing FASN (FASN / shFABP5) or MAGL (MAGL / shFABP5). (B) Total vector and shFABP5 cell flux at the primary tumour site, whole mouse, and femurs. (C) Total vector flux, FASN, and FASN / shFABP5 cells at the primary tumour site, whole mouse, and femurs. (D) Total vector flux, MAGL, and MAGL / shFABP5 cells at the primary tumour site, whole mouse, and femurs. Data was given as means ± SEM. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $**** \ p < 0.0001$ vs. vector. $\# \ p < 0.05$; $\#\# \ p < 0.01$; $\#\#\# \ p < 0.001$; $\#\#\#\#p < 0.0001$ represents FASN vs. FASN/shFABP5 or MAGL vs. MAGL/shFABP5; (n = 8) (Carbonetti et al., 2019).

The differences indicated by the researchers could be used to segregate prostate cancer patients into different clinical categories.

Furthermore, the identification of phosphorylation-dependent differences in the metastasis pathways could be used to provide personalized treatment to patients. The research into phosphorylation-dependent prostate cancer hallmarks offers a unique advantage to improve cancer medication by developing targeted therapies (Xu et al., 2016). However, it is essential to note that despite the promise to deliver more accurate and targeted treatment, the cost associated with these kind of tests are yet to be developed and require significant financial investment.

Cellular signalling networks have been used to develop biomarkers for cancer diagnosis. The advancement in molecular and proteomic techniques such as Quantitative Proteomics Signature Profiling (qPSP) and Paired Fuzzy SubNetworks (PFSNet), has enhanced network analysis in prostate cancer patients (Goh et al., 2019). In protein complexes, qPSP has been shown to increase the biological content of proteomic data by translating protein expressions into hit-rates. Additionally, qPSP can be used for the study of extremely heterogeneous clinical prostate cancer proteomics data, considering its capacity to differentiate phenotype groups even at small sample sizes and high noise robustness, as well as its improved summary statistics (Goh et al., 2015; Wang et al., 2017). Likewise, it has been observed that PFS-Net-identified subnetworks have greater accuracy over independently collected datasets compared to other approaches (Lim and Wong, 2014). Via network-based methods such as PFSNet and qPSP, researchers have noticed that low overlaps in patient data can emerge from a lack of sensitivity of the analytical methodology used, when considering the apparent overwhelming amount of heterogeneity amongst patients with prostate cancer (Fischer et al., 2019; Ho et al., 2020). Goh et al. (2019), reported that network-based analysis is more effective than protein analysis approaches in prostate cancer diagnosis. In general, network-based analysis provides more accuracy, precision, and sensitivity in diagnosis (Goh et al., 2019).

THE POTENTIAL USE OF EXOSOMES AS BIO-MARKERS

The analysis of biofluid has broadened the approaches applied to diagnose PCa. The involvement between extracellular vesicles in intracellular signalling provides a promising diagnostic strategy for PCa (Dhondt et al., 2020). According to Dhondt et al. (2020), these extracellular fluids can be used as biomarkers for diagnosis and prognosis of PCa. With the application of high throughput centrifugation and mass spectrometry techniques, extracellular vesicles have been extracted and analysed in urine from men with benign and malignant prostate tumours (Sequeiros et al., 2016; Dhondt et al., 2020). Further analysis of the proteome profile of the extracellular vesicles has helped to separate individuals with prostate cancer from those without the disease. The advances in proteome analysis of biofluids such as urine increase opportunities for diagnosis of the disease through non-invasive methods.

Extracellular vesicles are involved in intracellular communication where they carry a variety of cargo from the intracellular space to extracellular fluid. The vesicles may be involved in trafficking of cargo such as metabolites, non-coding RNA, and/or proteins (Nguyen et al., 2019). The molecular components of the extracellular vesicles can be used to examine the functionality of the parent cells, thus providing insight into the state of the cell (whether cancerous or
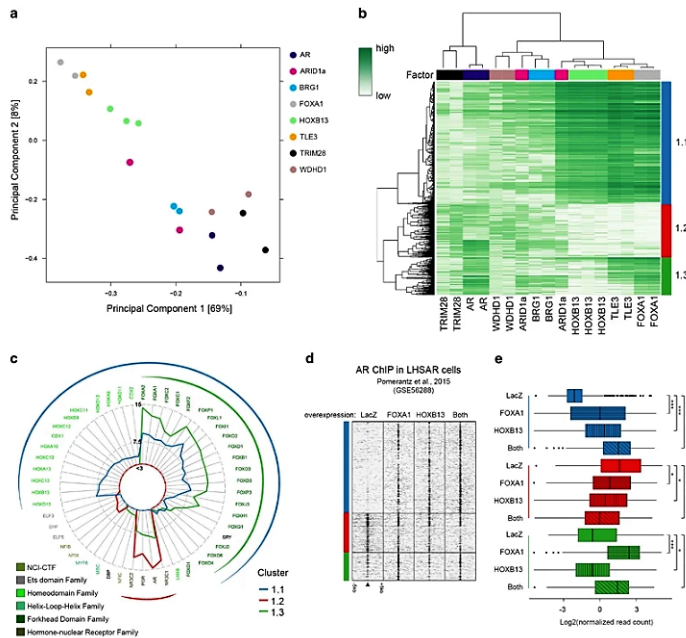
Figure 2: Clusters linked to carcinogenesis of prostate cancer were identified by application of both proteomic and genetic analysis. (A) Principal component analysis scores plot based on the read counts of each TF in AR-binding sites under R1881 conditions. (B) Heatmap of the Top 2000 regions with the most variable binding at AR sites of the TFs indicated. Based on the hierarchical clustering 3 clusters were identified. (C) Radar plot showing pattern enrichment in the Top 2000 variable regions (separated in the three clusters). The radii lengths are the absolute Z-score. The pattern colours correspond to the TF families. (D) Heatmap showing AR ChIP-seq signal (FPKM) in three clusters of LHSAR cells transduced with LacZ control, FOXA1, HOXB13 or both. Data is centred at AR peaks, depicting a 5-kb window around the peak. (E) Boxplot visualising the normalised AR signal (FPKM) at AR binding sites in clusters 1.1 (blue), 1.2 (red) and 1.3 (green). $p < 0.05$, * * * $p <$ 1e−16 (t-test) (Stelloo et al., 2018).

not) (Nguyen et al., 2019; Hornung et al., 2020). The use of extracellular fluid to analyse the cellular state is a new technique that is currently being considered for diagnosis and prognosis of prostate cancer. Hornung et al. (2020), analysed the exosome composition of VCaP cells and reported a significant resemblance between the extracellular vesicles and the parent cells. The findings reaffirmed the applicability of exosome analysis as a useful proteomic tool for prostate cancer diagnosis (Ishizuya et al., 2020).

PROTEOME ANALYSIS ENHANCE PERSONALISED TREATMENT

The use of biomarkers to enhance personalised prostate cancer treatment is rapidly gaining popularity. However, the majority of biomarkers currently available for cancer diagnosis and prognosis have not been validated and show poor reproducibility within a cohort of patients. This caveat hinders the application of these biomarkers in clinical settings. Thus,

research into the identification of robust novel biomarkers focuses on finding more effective molecular signatures for disease diagnosis. Kim et al. (2016), used Multiplexed SRM-MS to assess the effectiveness of biomarkers for the diagnosis of prostate cancer in urine samples. Out of 48 urine samples analysed, the researchers identified ANXA3, IDHC, PEDF, PRDX6, SERA, and TGM4 as potential biomarkers for use in the diagnosis of prostate cancer (Jadvar et al., 2020).

Proteome analysis of the bones among prostate cancer patients has revealed a significant heterogeneity. An analysis of bone metastasis demonstrated that there are more diverse proteins participating in cell cycle regulation compared to primary prostate tumours. Molecular changes such as alteration of processing of RNA and damages in DNA were identified (Zhang et al., 2019). Metabolic changes were also observed, such as oxidation of fatty acids and carbohydrate metabolism. In general, these metabolic alterations in bone metastasis were associated with cellular adhesion characteristics of the bone cells. These findings were indicative of the possibility to apply bone metastasis as a diagnostic measure for prostate cancer (Rodríguez-Blanco et al., 2018).

Multiple studies have focused on the total cell protein alterations in prostate cancer in the identification of novel biomarkers (Welton et al., 2016; Totten et al., 2018; Sinha et al., 2019). Moreover, the researchers emphasised the effectiveness of affordable proteomic analysis tools such as mass spectrometry. Compared to the expensive and rare genetic tools, including DNA/RNA sequencing approaches, it is rational to apply proteomic analysis (Latonen et al., 2018). Furthermore, integrative proteomic analyses are more precise and accurate compared to molecular examination of DNA methylation and gene analysis. Latonen et al. (2018) suggested that the application of more effective proteome analysis methods could speed up the process of personalised prostate cancer treatment.

PROTEOMIC PROFILING OF PROSTATE CANCER

The total protein profile in prostate cancer tissues is an essential tool for diagnosis and monitoring of disease progression (Zhou et al., 2019). The understanding of global alteration in the cellular protein profile may hasten the discovery of robust diagnostic methods and personalized treatment. However, the knowledge of changes in the protein components of prostate cancer cells is still limited (Zhang et al., 2018). Quantitative proteomic analysis to profile the total protein constituents of prostate cancer cells has been performed using transgenic mice (Zhang et al., 2018). Zhang et al. (2018) compared the total protein constituted of transgenic mice against the protein profile of wild type mice and reported 61 proteins that were differentially expressed between wild type and transgenic mice. Researchers suggest that bioinformatics tools combined with accurate proteomic tools can help leverage the power of the proteome profile in

cancerous cells to perform a precise diagnosis of prostate cancer (Pappireddi et al., 2019).

PROTEIN PROFILING AND PROSTATE CANCER DRUG DISCOVERY

Stelloo et al. (2018) analysed the androgen receptor protein profile using immunoassays. The researchers identified androgen receptor proteins among other interactors. Interestingly, most of the identified proteins were required for the progression of prostate cancer (Stelloo et al., 2018). Further analysis using chromatin immunoassays and sequence-based assays enabled the researchers to identify distinct sub-complexes of androgen receptor interactors. The complexes identified were selectively adapted to tumorigenesis. The findings reported by Stelloo et al. (2018) emphasised the importance of performing proteome analysis as a diagnostic approach for prostate cancer (Figure 2).

Prostate cancer chemotherapy involving the inhibition of androgen receptors expression can be investigated in order to improve treatment outcomes. However, the inhibition of androgen receptor expression requires an alteration in heat shock protein expression—a process that involves the identification of useful biomarkers. Robust predictive biomarkers may contribute to the strategy of personalised treatment. Stelloo et al. (2018) applied MS analysis to the expression of heat shock protein 90 in order to discover how its inhibition could be used to inhibit the proliferation of prostate cancer cells. The researchers reported eight proteins that were inhibited that belonged to the cell cycle regulation family. The findings provided insight into the application of proteome profile analysis to identify putative protein targets for drug discovery. In addition, the protein inhibition profiles were characteristic to some patients, which suggested the possibility of personalised treatment. Other studies (Garrido-Rodríguez et al., 2019; Singh and Sharma, 2020) have also reported the role of SWATH protein profiling tool in unveiling new drug targets. It is, therefore, imperative to use the protein profiling tools for the identification of putative drug target proteins in the process of prostate cancer drug discovery.

Coupling microscopy and laser capture microdissection (LCM) techniques have enabled scientists to target and extract specific cell types from tissues. A combination of LCM and LC/MS-MS has been used to remove proteins from prostate tissue (Staunton et al., 2016). The researchers reported high accuracy in identification and isolation of the target cells. The findings from the literature have demonstrated the applicability of protein profiling in drug target identification to enhance the specificity of newly developed cancer drugs while limiting cytotoxicity effects to non-target cells.

CONCLUSION

This literature review has reported evidence of the physiological processes involved in the proliferation of prostate cancer cells. Metabolic signalling and regulation play a central role in the aetiology of prostate cancer. Aberrant regulation of lipid metabolism due to abnormal expression of FASN enzyme has been widely documented in the literature. An abnormal increase in the expression of this enzyme has been associated with prostate cancer. Prostate cancer diagnosis and prognosis has been enhanced through the use of biomarkers. The literature search showed that numerous biomarkers had been recommended in the diagnosis of prostate cancer. However, the challenge is that the majority of the biomarkers are specific, limiting their application to research only. The use of prostate cancer biomarkers has also been extended to biofluid analysis of extracellular vesicles. These vesicles have been shown to contain protein profiles similar to their parent cells, thus demonstrating the application of non-invasive diagnostic tools in the diagnosis of prostate cancer. The literature search on the application of proteomic profiling has revealed a high potential for enhancing drug targeting during the drug development process. Profiling of total proteins in prostate cancer cells will help the drug development process optimise drug activity while limiting cytotoxicity to non-target cells. Future studies should focus on the incorporation of robust bioinformatics tools for more effective drug modelling and target optimisation.

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