Original article

Protein patterns in Nigerian men with prostate cancer

***Adeola Oluboyo[1], Samuel Meludu[2], Charles Onyenekwe[1] , Bernard Oluboyo[3], Ikemefuna Uzochukwu[4], Chidi Oranusi[5], Timothy Mbaeri[5]**

[1]Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

[2]Department of Human Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

[3]HIV Care Department, Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria

[4]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

[5]Department of Surgery, Nnamdi Azikiwe University Teaching Hospital, Nnewi Anambra State Nigeria

Corresponding author : Adeola Oluboyo

Abstract

The increasing prevalence of prostate cancer is a challenge. Early detection and diagnosis of prostate cancer are vital in its management. Therefore the present study was designed to determine proteins that may be expressed in serum and urine of prostate cancer subjects. A total of fifty men diagnosed with prostate cancer and fifty healthy men (controls) were recruited for the study. Blood and urine specimen were collected from the subjects for the analysis of serum and urinary proteins using sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins (kiloDalton) detected exclusively in the serum of prostate cancer subjects were p6.37 (8%), p9.18 (14%), p12.38 (12%), p13.38 (20%), p14.26 (6%), p16.0 (8%), p17.28 (4%), p22.36 (18%), p32.36 (30%), p34 (16%), p55.17 (6%), p64.26 (6%), p73.09 (8%), p91.08 (12%), p93.21 (4%), p94.06 (6%), p94.47 (12%) and p99.19 (10%) while p20.22 (8%) and p46.22 (8%) were detected in the urine of prostate cancer subjects. None of these proteins were expressed in the control subjects. The uses of these proteins alongside the estimation of the currently used markers may provide a strong lead way for the early detection and diagnosis of prostate cancer.

Key words : prostate cancer, electrophoresis, serum protein, urine proteins

Introduction

The increasing prevalence of prostate cancer is a challenge. The prevalence of prostate cancer in Nigeria was formerly put to be about 15.7% [1,2]. It was also reported that Prostate Cancer is the most common cancer in Nigerian males; having overtaken lung cancer[3]. However, another study carried out later reported that the incidence of Prostate cancer in Nigeria is 18.2% and prostate cancer accounts for about 9.6% of all mortality from Cancer cases in Nigeria[4]. The trend at which prostate cancer is growing is alarming and attention needs to be paid to this. The current screening method for prostate cancer relies on a combination of Prostate specific antigen (PSA) assay and a Digital Rectal Examination (DRE) while biopsy is done to confirm it if there is suspicion of cancer. It is well recognized that the serum prostatic specific antigen (PSA) as a biomarker of prostate disease may not always suggest the presence of cancer and conversely will rise in the

presence of benign prostatic hyperplasia (BPH), prostatitis and after urethral manipulation[5,6]. Early detection and diagnosis of prostate cancer are vital in the management especially in subjects who have little or no symptoms. It has been shown that the use of multiple biomarkers in screening and diagnosis will be necessary to produce better results[7]. It has also been shown that the development of cancer is associated with alterations in numbers and functions of immune cells (proteins) in the peripheral circulation and especially at the sites of tumor progression[8]. Thus, the present study was designed to determine serum and urine proteins that may be expressed in serum and urine of prostate cancer subjects.

Materials and methods

Fifty prostate cancer subjects with mean age of 69 ± 7 years who were attending the Surgical Out-Patients' Clinic of Nnamdi Azikiwe University Teaching Hospital, South East Nigeria were investigated after they had been diagnosed of prostate cancer between September 2011 and June 2012. Fifty apparently healthy subjects without prostate cancer with mean age of 52±13 years served as controls. Five milliliter of venous blood was drawn aseptically into dry plain containers after the consent of the subjects had been sought and obtained. The serum was separated immediately after clot retraction and stored at -20^0C until the time of assay. Urine specimens were also collected from the subjects into sterile universal containers. Proteomic pattern in serum and urine were detected using SDS-PAGE electrophoresis for identification of proteins. The gels were scanned using software known as UNSCAN-IT which contains features for analyzing electrophoresis gels. The gel images were analyzed and the detected spots were manually edited for more accuracy. The digitized data was used to determine the molecular weights of the proteins. Molecular weight marker from Invitrogen Corporation (SeeBlue® Plus2 Pre-Stained Standard) was used as standard Molecular weight marker. The molecular weights were used to identify and compare the differentially expressed proteins between prostate cancer subjects and control subjects. The protein names were obtained from Protein Information Resource (PIR), Protein Data Bank (PDB) and National Center for Biotechnology Information (NCBI).

Results

The data were presented in simple percentages. The results of the proteomic analysis were expressed in kiloDalton (kDa). The molecular weights and the names of the proteins detected from protein electrophoresis are shown on Tables 1, 2 and 3.

Discussion

The proteins reported in this study were detected exclusively in serum and urine of prostate cancer subjects. The proteins have been linked with the modulation of the immune cells and they have been implicated in cancer. For instance, p12.38 and p13.38 [immunoglobulin (Ig)] have been shown to play essential roles as antibody in blood and body fluids and serve as protective protein produced by the immune system in response to foreign antigens[9]. This emphasizes the key role of humoral immunity in control of cancer progression and has major implications for determining prognosis of patients with cancer[10]. Also p34 (MHC class I molecules) binds peptides that have been processed by APC and present them to CD8+ T lymphocytes for elimination. This implies that expression of MHC class I molecules on tumours is also mandatory for an effective T cell response against cancer. MHC and HLA expression are important in presenting tumour

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antigens to CD8+ T lymphocytes for destruction. P32.36 (Ubiquitin thioesterase and trinucleotide repeat containing gene 18 protein) is a regulatory protein which marks other targeted proteins for degradation. P22.36 (S100 calcium binding protein also known as Annexin A2) mediates angiogenesis and enhances tumor growth and metastasis[11]. Annexin2 has been suggested to be capable of modulating key events in tumor progression mainly those involving invasion, metastasis, and drug resistance. P7.33 (Heat shock protein beta-7, Soluble interleukin-4 receptor subunit alpha, Alternative protein ALPK1) and p13.05 (Ribonuclease pancreatic, SEC14-like protein 1) were detected in the urine of prostate cancer subjects. Heat shock proteins (Hsps) have been shown to be overexpressed in a wide range of human cancers and are implicated in tumour cell proliferation, differentiation, invasion, metastasis, death, and recognition by the immune system[12,13]. Heat-shock proteins, especially Hsp70 are involved in binding antigens and presenting them to the immune system for elimination[14]. SEC14-like 2 genetic variants have been associated with breast size and this also influence breast cancer risk. Although their specific functions are not well established, they have been implicated in the regulation of subcellular localization of their host proteins.

P1.67 [Neprilysin or endopeptidase, Nuclear receptor coactivator- 3 (NCOA3) or steroid receptor coactivator-3 (SCR-3)] was also detected in urine of prostate cancer subjects. Neprilysin has been shown to play a regulatory role on the peptides that are involved in the physiological mechanisms of mammalian nervous, cardiovascular and immune systems[15]. SCR-3 protein is a multifunctional protein that plays an important role in mammary gland growth, development, and tumorigenesis[16]. In their study on the effects of steroid receptor coactivator-3 (SCR-3) on the immunosuppression accompanied with systemic inflammatory response syndrome (SIRS), it was demonstrated that the absence of SCR-3 protein would aggravate immunosuppression with an attendant invasion of the host by tumour antigen[17]. These proteins may serve as markers if detected in serum of apparently normal individuals since they were not detected in control subjects.

The study concluded that these proteins: p6.37, p9.18, p12.38, p13.38, p22.36, p32.36 and p34 may serve as markers for the detection of prostate cancer in apparently healthy individuals. The uses of these proteins alongside the estimation of the currently used markers may provide a strong lead way for the early detection and diagnosis of prostate cancer.

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Table 1 : Type and percentage prevalence of proteins (<30kDa) in serum of prostate cancer subjects

Proteins	Proteins names	Percentage
p6.37	Survivin isoform, Endoplasmic reticulum-Golgi intermediate compartment protein 2, Transmembrane protein 229B,Sodium channel and clathrin linker 1	4 (8%)
P9.18	Zinc finger protein (Fragment),Ly6/neurotoxin-like protein 1 (Fragment)	7(14%)
p12.38	Ig lambda chain V region 4A,Phosphoacetylglucosamine mutase	6 (12%)
p13.38	Ig kappa chain V-IV region precursor DKFZp686G1626 Serine/arginine repetitive matrix protein 2	10(20%)
P14.26	cDNA FLJ10342 fis, clone NT2RM2000837 (Fragment), Putative uncharacterized protein DKFZp547M202 (Fragment), Tetratricopeptide repeat protein 21A	3(6%)
P16.0	Serine palmitoyltransferase 3, Nucleosome assembly protein 1-like 4	4 (8%)
P17.28	cDNA FLJ34521 fis, clone HLUNG2007041, Oligoribonuclease, mitochondrial	2 (4%)
P22.18	Peptidyl-prolyl cis-trans isomerase FKBP11 precursor, Phosphatidylethanolamine-N-methyltransferase-like protein, Zinc finger imprinted 2, Mucolipin-1	4 (8%)
P22.36	S100 calcium binding protein A10 (Annexin II ligand, calpactin I, light polypeptide (P11), isoform CRA_b cDNA FLJ52136, highly similar to NEDD4 family-interacting protein 2, cDNA FLJ00332 protein, Signal-regulatory protein beta-2	9(18%)

N =50

Table 2 : Type and percentage prevalence of proteins (>30kDa) in serum of prostate cancer subjects

Proteins (kDa)	Proteins names	Percentage
p32.36	Ubiquitin thioesterase, Trinucleotide repeat-containing gene 18 protein	15(30%)
p34	MHC class I antigen	8(16%)
P38.76	EHMT1 protein	4 (8%)
P55.17	cDNA FLJ56942, highly similar to Latrophilin-3	5(10%)
P57.48	Neuronal acetylcholine receptor subunit alpha-3 precursor	8(16%)
P64.26	Transmembrane anterior posterior transformation protein 1 homolog or Cytomegalovirus partial fusion receptor, cDNA FLJ76695, highly similar to Homo sapiens acyl-CoA synthetase medium-chain family member 2	3(6%)
P73.09	PR domain zinc finger protein 5	4 (8%)
p91.08	DnaJ homolog subfamily C member 10 precursor, cDNA FLJ58448, highly similar to Bromodomain-containing protein 8	6 (12%)
p93.21	cDNA FLJ55570, highly similar to Protein SMG7	2 (4%)
p94.47	ANK repeat and pH domain-containing protein 1 (Centaurin-gamma-2)	6(12%)
p99.19	Cytosolic phospholipase A2 epsilon	5(10%)

N =50

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Table 3: Type and percentage prevalence of proteins in urine of prostate cancer subjects

Proteins	Proteins names	Percentage
P1.67	Nuclear receptor coactivator 2, Neprilysin	3(6%)
P5.07	A/G-specific adenine DNA glycosylase, Rho GTPase-activating protein 7, HCG1813589, isoform CRA_a	2 (4%)
P7.33	Heat shock protein beta-7, Soluble interleukin-4 receptor subunit alpha(IL4R nirs) variant1), Alternative protein (ALPK)1	4 (8%)
P13.05	Keratin, type II cytoskeletal, Zinc finger protein 313, isoform CRA_b, Ribonuclease pancreatic, SEC14-like protein 1	2 (4%)
P20.22	cDNA FLJ51878, highly similar to Creatine kinase	4 (8%)
P55.8	Mab-21 domain-containing protein 2, Lysosomal Pro-X carboxypeptidase precursor, cDNA, FLJ95856, highly similar to Homo sapiens fucosyltransferase 11	2 (4%)

N =50

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