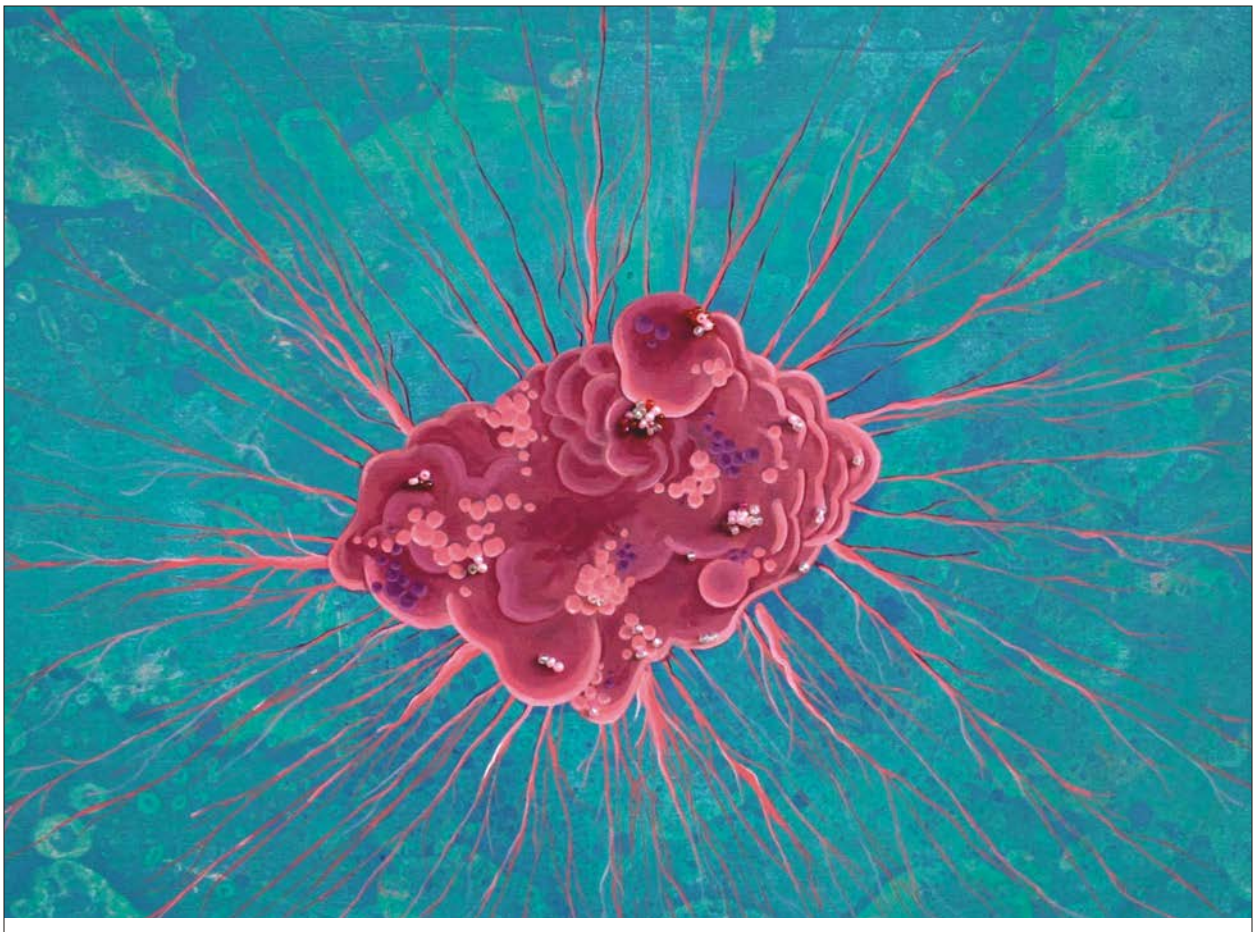

International Journal of Cancer Research

ISSN 1811-9727

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Cancer Research

ISSN 1811-9727
DOI: 10.3923/ijcr.2016.82.91

Research Article
HSPD1, HSPB1 and VDAC1 are Over-expressed in Invasive Ductal Carcinoma of the Breast

[1]Mutiu A. Alabi, [2]Olugbenga O. Adebawo, [3]Oluwole A. Daini, [4]Stella B. Somiari and [5]Richard I. Somiari

[1]Bioresources Development Centre, National Biotechnology Development Agency, Ogbomosho, Nigeria
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Remo Campus, Ikenne, Nigeria
[3]Department of Biochemistry, School of Science, Federal University of Technology, Akure, Nigeria
[4]Biobanking and Biospecimen Science Research Unit, Windber Research Institute, Windber, Pennsylvania, USA
[5]Functional Genomics and Proteomics Unit, ITSI-Biosciences LLC, 633 Napoleon Street, Johnstown, Pennsylvania, USA

Abstract

Background and Objectives: The initiating steps and precise pathway of breast tumorigenesis are poorly understood and it is unclear if Ductal Carcinoma *In Situ* (DCIS) progresses to invasive ductal carcinoma (IDCA) of the breast. This study was undertaken to identify proteins that are differentially expressed between IDCA and DCIS and that may predict the invasive potential of breast tumors. **Methodology:** It is utilized that the two-dimensional difference in gel electrophoresis technology (2D-DIGE) and tandem mass spectrometry (LC-MS/MS) to perform proteomic analysis of IDCA (MCF-7 and BT-474) and DCIS (HCC-1500 and HCC-38) cell lines. **Results:** Identified 10 proteins that were differentially expressed between IDCA and DCIS ($\geq$2-fold difference; $p \leq 0.05$) and classified the proteins according to their Gene Ontology (GO). Out of these proteins, 60 kDa mitochondrial heat shock protein (HSPD1), Heat Shock Protein Beta 1 (HSPB1) and the voltage-dependent anion-selective channel protein 1 (VDAC1) are over expressed in IDCA compared to DCIS. **Conclusion:** The functional role of the differentially expressed proteins suggests that they may serve as biomarkers for identification of tumors with invasive potential.

Key words: Breast cancer, heat shock protein, two-dimensional difference gel electrophoresis, mass spectrometry

Received: December 04, 2015 Accepted: February 19, 2016 Published: March 15, 2016

Citation: Mutiu A. Alabi, Olugbenga O. Adebawo, Oluwole A. Daini, Stella B. Somiari and Richard I. Somiari, 2016. HSPD1, HSPB1 and VDAC1 are over-expressed in invasive ductal carcinoma of the breast. Int. J. Cancer Res., 12: 82-91.

Corresponding Author: Mutiu A. Alabi, Bioresources Development Centre, National Biotechnology Development Agency, Ogbomosho, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Breast cancer is the most commonly diagnosed cancer in women and it accounts for a significant number of cancer–related deaths in women in Nigeria[1,2]. According to the American Cancer Society[3], breast cancer ranks second as the cause of cancer death in women in America. It was estimated that 234,190 new breast cancer cases will be diagnosed in the US in 2015 and about 40,730 were expected to die from the disease[3].

There are different types of breast cancer. Cancer cells that remain confined to the lobule and the ducts are called *in situ* or non-invasive. They are sometimes also referred to as pre-cancers in recognition of the fact that these cells have not yet gained the ability to spread to other parts of the body, which is the feature that most people associate with cancer. The non-invasive breast cancers are ductal carcinoma *in situ* also called intraductal carcinoma and lobular carcinoma *in situ* and accounts for about 10% of all cases[4].

An invasive cancer is one, where the cells have moved outside the ducts and lobules into the surrounding breast tissue. Infiltrating ductal carcinoma, an invasive cancer, penetrates the wall of a duct and is the most common form of breast cancer constituting about 70% of all cases. Infiltrating lobular carcinoma, also an invasive cancer spreads through the wall of the lobule. It accounts for about 8% of all breast cancer cases. The breast can be divided into 4 quadrants excluding the nipple and cancer is most frequent in the upper outer quadrant of the breast. Each quadrant has its rate and percentage of occurrence[4].

Ductal carcinoma *in situ* lies along a broad range of related qualities of preinvasive lesions originating within normal breast tissue with histologic progression from atypical hyperplasia to invasive breast cancer[5]. Although, the initiating steps and precise pathways of breast tumorigenesis remain poorly defined, it appears that nearly all invasive breast cancers arise from *in situ* carcinomas. Hence, this study was undertaken to use proteomics to identify proteins that are differentially expressed in invasive ductal carcinoma (IDCA) when compared with Ductal Carcinoma *In Situ* (DCIS). Differentially expressed proteins may serve as biomarkers differentiating IDCA from DCIS and most importantly find utility in predicting the onset of invasion of breast cancer.

MATERIALS AND METHODS

Human breast cancer cell lines: Four human breast cancer cell lines namely MCF-7, HCC-38, HCC-1500 and BT-474 were obtained from American Type Cultural Collection (ATCC), Manassas, VA, USA and used for the study. The cell lines were cultured and maintained at 37.0°C in the presence of 95% atmospheric air and 5% carbon dioxide (CO_2) using a complete growth medium (MCF-7 in ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003, BT-474 in ATCC Hybri-Care Medium; Catalog No. 46-× and HCC-38 and HCC-1500 in ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001) supplemented with fetal bovine serum. All tissue culture experiments were performed at the Windber Research Institute, Windber, PA, USA. At the end of cell culture, the cells were harvested and re-suspended in ToPI-DIGE buffer (ITSI-Biosciences Johnstown, PA, USA) and stored at -80°C until analyzed.

Two-dimensional difference gel electrophoresis (2D-DIGE): The samples were analyzed by 2D-DIGE and tandem mass spectrometry. Briefly, total protein was isolated from each cell line using the ToPI-DIGE kit, total protein concentration determined using the ToPA protein assay[6]. For 2D-DIGE, the minimal-labeling protocol previously described[7] was used to label 50 µg of total protein from each sample using 200 pmole of Cy3 or Cy5 fluorescent dyes. Additionally, an equal aliquot of protein from all samples in the study were pooled and labeled with Cy2 dye. The Cy2 labeled pooled sample was added to each gel and used as the universal internal control (U) to allow for the quantitative comparison of all samples. The Cy2, Cy3 and Cy5 labeled samples in addition to 175 µg of unlabelled protein were mixed and loaded on a single 24 cm IEF strip, pH 3-10 NL for the 1st dimension separation using the Ettan IPGphor II (GE Healthcare, Piscataway, NJ). The strips were rehydrated in the presence of the samples for 12 h at 30 V and then focused for a total of 65,000 V h[6]. For 2nd dimension separation, each focused strip was loaded onto a 24×20 cm, 12.5% SDS-PAGE gel and run for 4 h. After 2D-DIGE (1st and 2nd dimension separations) the gels were scanned at three wavelengths with a DIGE enabled variable mode digital scanner (Typhoon Trio; GE Healthcare Piscataway, NJ) to capture the Cy3, Cy5 and Cy2 signals. Subsequently, each gel was stained with Sypro Ruby, scanned and a pick list of candidate proteins generated as previously described[6].

Image analysis: The captured gel images were analyzed using the difference in gel analysis (DIA) module of DeCyder software (version 6.0; GE Healthcare Lifesciences, Piscataway, NJ, USA).

The normalized spot volumes generated in DeCyder that showed ≥2-fold difference in abundance between the IDCA and DCIS cell lines ($p \leq 0.05$) were considered candidate differentially expressed proteins. After DIA, each gel was stained with Sypro Ruby to allow accurate identification and matching of spots to be picked[7].

Candidate protein spot processing: Candidate protein spots were picked using the Ettan robotic spot picker (GE Healthcare Lifesciences, Piscataway, NJ, USA) and in-gel digested using the Ettan robotic spot digester. Briefly, the spots were picked into 96-well plates and digested with trypsin overnight (15-16 h) at room temperature. The digested samples were extracted in 50 µL of 50% acetonitrile/0.1% formic acid prior to mass spectrometry[6].

Protein identification by mass spectrometry: Protein identification was performed using a thermo scientific surveyor High-Performance Liquid Chromatography (HPLC) system connected to an LCQ DECA XP plus iontrap mass spectrometer with a nanospray ionization source (ThermoFinnigan, San Jose, CA). Mass spectrometry conditions were as previously described[6].

Database search: Each acquired MS/MS spectrum was searched against the international protein index database (human) version 3.72 using the SEQUEST search algorithm. Proteins were identified when two or more unique peptides had X-correlation scores above 1.5, 2.0 and 2.5 for respective charge states of +1, +2 and +3 and the delta CN score was greater than 0.1. Each candidate ID derived from the above search was then manually examined in the SwissProt database to eliminate redundancy of synonymous proteins. A protein's name and accession number were reported based on SwissProt except for proteins that are only deposited in the NCBI database.

Gene ontology: Differentially expressed proteins identified by 2D-DIGE were further classified according to their Gene Ontology (GO) using NCBI and KEGGS databases[8]. The GO provides a controlled vocabulary to describe proteins in terms of Molecular Function (MF), Cellular Component (CC) and Biological Process (BP).

Statistical analysis: The t-test was used to determine the statistical significance of the difference in protein expression in IDCA compared with DCIS. For all comparisons, the level of significance was set at $p \leq 0.05$.

RESULTS

Average protein concentrations: The average protein concentrations of the cell lines (MCF-7, HCC-38, HCC-1500 and BT-474) were as presented in Table 1. The BT-474 cell line had the highest protein concentration (53,000 µg mL^{-1}) while HCC-38 cell line had the lowest protein concentration (7,360 µg mL^{-1}).

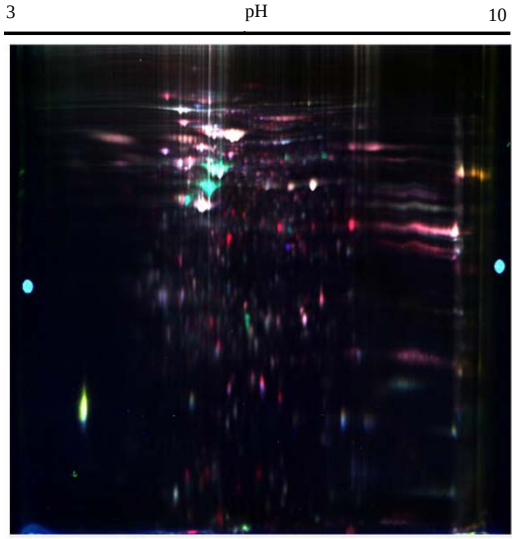

Fig. 1: 2D-DIGE gel image showing candidate protein spots from MCF-7 (Green) vs: HCC-38 (Red) cells

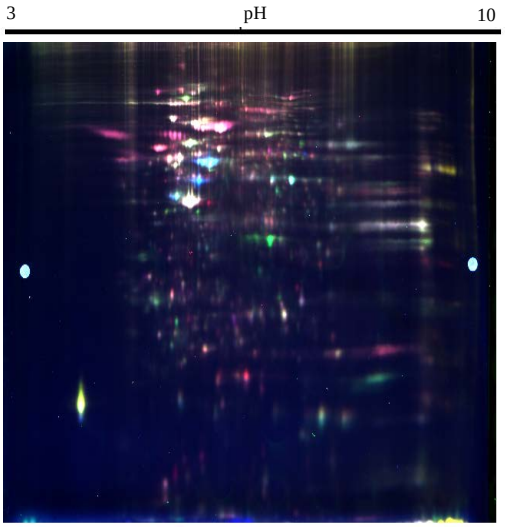

Fig. 2: 2D-DIGE gel image showing candidate protein spots from HCC-1500 (Green) vs: BT-474 (Red)

Table 1: Average protein concentrations of the cell lines

Sample name	Protein concentration (µg mL^{-1})
MCF-7	24,520
HCC38	7,360
HCC-1500	12,210
BT-474	53,000

Identifying proteins differentially expressed in breast tumor cell lines: The DeCyder software was used to analyze the 2D-DIGE gel images using the difference in gel analysis workspace. A total of 18 candidate protein spots were identified as differentially expressed between HCC-38 vs. MCF-7 (Fig. 1) and between HCC-1500 vs. BT-474 (Fig. 2) by a factor of at least 2-fold (Table 2).

Table 2: Average ratio of protein expression between MCF-7 vs. HCC-38 and BT-474 vs. HCC-1500 cell lines

Spot No.	Symbol	Protein name	MCF-7/HCC-38 average ratio	p-value	BT-474/HCC-1500 average ratio	p-value
355	HSPD1	60 kDa heat shock protein and mitochondrial	-1.19	0.046	-2.00	0.021
509	HSPD1	60 kDa heat shock protein and mitochondrial	-2.09	0.010	-3.53	0.001
523	TPI1	Triosephosphate isomerase	-	-	6.12	0.023
573	ACTB	Actin and cytoplasmic 1	-	-	1.84	0.023
614a	G6PD	Glucose-6-phosphate 1-dehydrogenase	8.77	0.014	-	-
614b	FGB	Fibrinogen beta chain	8.77	0.014	-	-
614c	ALDH1A3	Aldehyde dehydrogenase family 1 member A3	8.77	0.014	-	-
614d	MCCC2	Methylcrotonoyl-CoA carboxylase beta chain and mitochondrial	8.77	0.014	-	-
638	LASP1	LIM and SH3 domain protein 1	8.77	0.014	2.79	0.003
639a	VDAC1	Voltage-dependent anion-selective channel protein 1	1.66	0.021	-	-
639b	SCYL2	SCY1-like protein 2	1.66	0.021	-	-
677a	ANXA5	Annexin A5	-1.13	0.660	-	-
677b		cDNA FLJ56307	-1.13	0.660	-	-
695a	HSPB1	Heat shock protein beta-1	1.38	0.084	1.83	0.008
695b	TPI1	Triosephosphate isomerase	1.38	0.084	1.83	0.008
699a	VDAC1	Voltage-dependent anion-selective channel protein 1	-6.53	0.003	-1.05	0.320
699b	HSPB1	Heat shock protein beta-1	-6.53	0.003	-1.05	0.320
704	PSMD7	26S proteasome non-ATPase regulatory subunit 7	-	-	2.20	0.016

Protein identification by mass spectrometry: The selected protein spots were excised, in-gel digested with trypsin and analyzed by LC-MS/MS. The raw data files were used to search against the Swiss-Prot human database using Mascot as a primary database search algorithm. Expasy Aldente was used as a complementary algorithm for additional confirmation to reduce the possibility of false positive identification. Agreement between the apparent MW and pI observed on the 2D-DIGE gel and the theoretical values of the identified proteins provided additional support for positive identification. Eleven proteins of interest were identified in the HCC-38 vs. MCF-7 comparison (Fig. 3) and 13 were identified in the HCC-1500 vs. BT-474 comparison. Out of these, 10 proteins showed statistically significant difference in spot volume ($\geq$2-fold) between the DCIS and IDCA cell lines (Table 3). Three proteins out of the 10 proteins showed over expression in IDCA cell lines when compared with DCIS cell lines. Some of the identified proteins may exist in multiple forms, as they were identified in spots picked from different sections of the gel (Table 4 and Fig. 4). It is unclear as to whether these proteins exist in multiple forms due to biological differences or processing influences, such as carbamylation, a common modification when using urea buffer, which causes shifts in the isoelectric point of the protein.

Gene ontology classification: The proteins of interest were categorized using NCBI and KEGGS databases to determine their molecular function, cellular component and biological process (Table 4). The most significant pathway associated with the differentially expressed proteins identified was the apoptosis signaling pathway.

DISCUSSION

Proteomics is increasingly being used to identify proteins that show differential expression in cancer[9]. Such protein alterations may eventually contribute to increasing this understanding of cancer pathogenesis as well as aid in the development of effective strategies for cancer diagnosis and treatment.

The proteins identified in this study span a wide range of functions and if validated may have value as diagnostic and prognostic biomarkers for breast cancer. Among the proteins identified were 60 kDa heat shock protein, mitochondrial (HSPD1), Heat Shock Protein Beta-1 (HSPB1) and voltage-dependent anion-selective channel protein 1 (VDAC1), which showed increased expression in IDCA cell line when compared with DCIS cell line.

The proteomic analysis revealed that the expression of 60 kDa HSPD1 was increased in IDCA cell lines (Table 2 and 4). The observation that HSPD1 is over expressed in IDCA is of interest and deserves further studies to determine if the overexpression is a result of the induction or inhibition of genes and/or degeneration or modification of proteins during carcinogenesis.

The HSPD1 is constitutively expressed under normal conditions and its expression is induced by stressful conditions such as heat shock, mitochondrial damage and mtDNA depletion1[0-14]. The overexpression of HSPD1 in IDCA breast cancer cell lines suggests that this protein might play a different role in breast carcinogenesis[15,16]. Paepe[15] and Cappello *et al.*[17] reported that elevated expression of HSPD1 may have a protective role against cancer

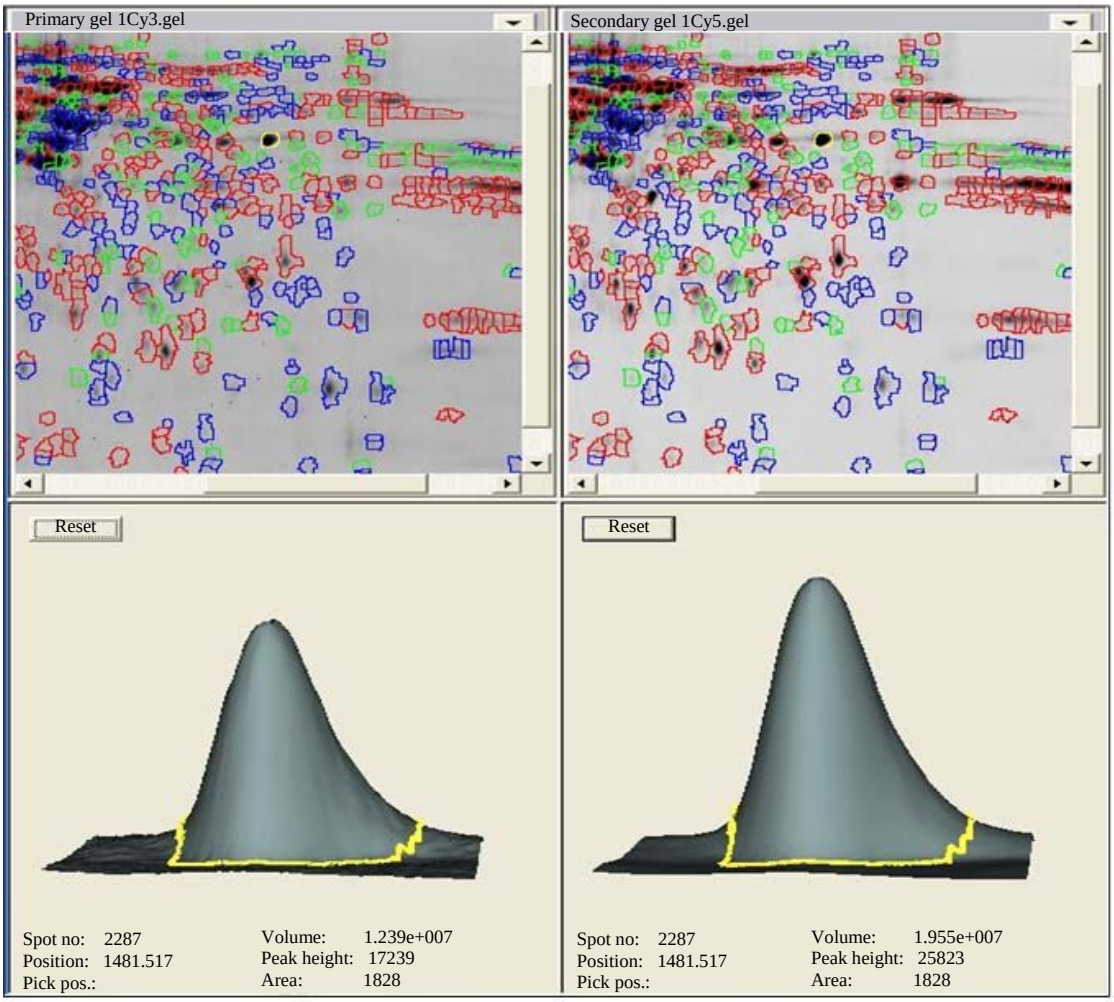

Fig. 3: Representative simulated 3D images of candidate protein spots differentially expressed between MCF-7 (Cy3) and HCC-38 (Cy5)

Table 3: List of proteins differentially expressed between IDCA and DCIS

Spot No.	Symbol	Protein name	Accession No.	MW	pI	Peptides matched	*Percent sequence coverage
355	HSPD1	60 kDa heat shock protein, mitochondrial	P10809	61017.4	5.59	6	13.96
509	HSPD1	60 kDa heat shock protein, mitochondrial	P10809	61017.4	5.59	10	32.29
523	TPI1	Triosephosphate isomerase	P60174	27110.1	8.24	5	24.10
573	ACTB	Actin, cytoplasmic 1	P60709	41710.7	5.18	1	4.80
614a	G6PD	Glucose-6-phosphate 1-dehydrogenase	P11413	63787.3	6.44	3	9.27
614b	FGB	Fibrinogen beta chain	P02675	55893.3	8.23	3	6.92
614c	ALDH1A3	Aldehyde dehydrogenase family 1 member A3	P47895	56073.8	6.98	3	7.81
614d	MCCC2	Methylcrotonoyl-CoA carboxylase beta chain, mitochondrial	Q9HCC0	61295.4	7.51	2	5.68
638	LASP1	LIM and SH3 domain protein 1	Q14847	29699.2	6.67	2	10.34
639a	VDAC1	Voltage-dependent anion-selective channel protein 1	P21796	30754.6	8.86	7	31.80
639b	SCYL2	SCY1-like protein 2	Q6P3W7	103643.4	8.22	1	1.94
677a	ANXA5	Annexin A5	P08758	35915.4	4.78	2	6.56
677b		cDNA FLJ56307	B4DPD5	35159.5	5.30	1	4.87
695a	HSPB1	Heat shock protein beta-1	P04792	22769.5	5.97	5	34.15
695b	TPI1	Triosephosphate isomerase	P60174	27110.1	8.24	3	17.27
699a	VDAC1	Voltage-dependent anion-selective channel protein 1	P21796	30754.6	8.86	9	32.86
699b	HSPB1	Heat shock protein beta-1	P04792	22769.5	5.97	5	35.12
704	PSMD7	26S proteasome non-ATPase regulatory subunit 7	P51665	37003.5	6.31	1	3.40

*Sequence coverage (%) and No. of peptides identified with #1%, PI: Protease inhibitor and MW: Molecular weight

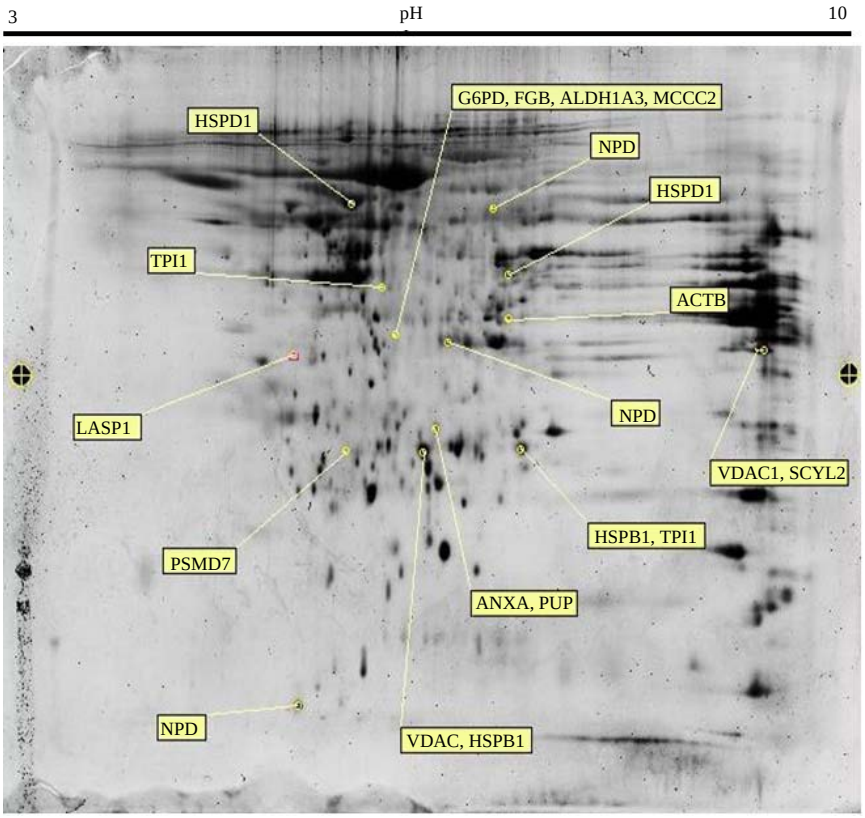

Fig. 4: Location of 14 differentially expressed proteins identified by mass spectrometry

Table 4: Expression pattern and gene ontology of differentially expressed protein in IDCA/DCIS cell lines

Protein name	Pattern	Biological process	Cellular components	Molecular function
60 kDa heat shock protein mitochondrial	↑	ATPase activity, DNA replication and chaperone	Mitochodrial inner membrane and matrix and cell surface	Protein folding and RNA degradation and apoptosis
Triosephosphate isomerase	↓	Carbohydrate metabolism and fatty acid anabolism	Cytosol, nucleus and soluble fraction	Isomerase activity and protein binding
Glucose-6-phosphate 1-dehydrogenase	↓	NADPH regeneration, pentose phosphate pathway and carbohydrate metabolism	Cytoplasm and internal side of plasma membrane	NADP or NADPH binding and glucose binding
Fibrinogen beta chain	↓	Blood coagulation, platelet activation and degranulation and response to calcium ion	Cell cortex, external side of plasma membrane and platelet alpha granule	Cheperone binding, protein binding and receptor binding
Aldehyde dehydrogenase family 1 member A3	↓	Positive regulation of apoptosis and retinoic acid biosynthesis	Cytoplasm	NAD binding, aldehyde dehydrogenase (NAD) and (NADP) activity
Methylcrotonoyl-CoA carboxylase beta chain, mitochondrial	↓	Branched chain amino acid catabolism and leucine catabolism	Mitochodrial matrix	ATP binding, ligase activity and methylcrotonoyl-CoA carboxylase activity
LIM and SH3 domain protein 1	↓	Ion transport	Cell cortex, cortical actin cytoskeleton and cytoplasm	Actin binding, ion transmembrane transpoter activity and protein binding
Voltage-dependent anion-selective channel protein 1	↑	Anion transport and apoptosis	Membrane, mitochodrial inner and outer membrane	Porin activity, protein binding and voltage-gated anion channel activity
Heat shock protein beta-1	↑	Anti-apoptosis, cell death, response to heat, virus and unfolded protein	Cytoplasm, cytoskeleton, nucleus and plasma membrane	Protein binding and ubiquitin binding
26S proteasome non-ATPase regulatory subunit 7	↓	Regulation of apoptosis and DNA damage repair	Proteasome complex	Protein binding

↑: Overexpressed and↓: Underexpressed

development (i.e., the blockade of apoptotic machinery that usually takes place during cancer progression).

The involvement of HSPD1 in the process of apoptosis and tumorigenesis is still in dispute[12,15,16]. An antiapoptotic effect of HSPD1 and down-regulation of HSPD1 have been reported in cardiac myocytes and bronchial cancer[17]. On the other hand, overexpression of HSPD1 has been reported in prostate and ovarian carcinomas and myeloid leukemia[15,17,18]. Moreover, recent studies showed upregulation of HSPD1 during carcinogenesis of the large bowel and the uterine exocervix[15,17,18].

The results of the study work revealed overexpression of Heat Shock Protein Beta-1 (HSPB1) in IDCA breast cancer cell lines. The HSPB1 belongs to the family of small stress proteins that are constitutively abundant and ubiquitously present in cells[12,14,15]. The HSPB1 regulates apoptosis through its ability to interact with key components of the apoptotic-signaling pathways[15,16]. Changes in the intracellular redox balance and production of reactive oxygen species initiate the apoptotic cascade through changes in the mitochondria and release of pro-apoptotic factors[14,15]. The HSPB1 can maintain both the redox homeostasis and mitochondrial stability in the cell[14].

The HSPB1 helps protect cells under adverse conditions such as infection, inflammation, exposure to toxins, elevated temperature, injury and disease[10]. Heat shock proteins block signals that lead to programmed cell death or apoptosis[10,14]. Up-regulated expression of HSPB1 has been reported in several cancers such as ovarian cancer, renal cancer, various leukemias, bladder cancer, etc.[19-22]. The overexpression of HSPs in tumorous tissues has been implicated to have prognostic value in patients with ovarian, renal and bladder cancer[15,20,23,24].

Recently, there have been reports that high HSPB1 expression levels are associated with a poor prognosis for specific cancers including gastric, liver and prostate carcinomas and osteosarcomas[10,12,14,15]. Furthermore, expression of HSPB1 in primary breast cancers is associated with a short survival for node-negative patients and increased HSPB1 expression levels have been found in highly metastatic variant breast cancer cells[10,15,23,25]. However, osteolytic bone metastases of human breast cancer cells are reduced by HSPB1 overexpression[26]. It is suggested that overexpression of HSPB1 may render tumors more resistant to some commonly used chemotherapeutic agents that induce apoptosis[27].

The HSPB1 is highly expressed in Her2-positive tumors[10,28]. The HSPB1 is one of the downstream effectors of p38 MAP kinase-mediated matrix metalloproteinase type 2 activation and is a modulator of Stat3-regulated apoptosis[29,30]. The Ser78 phosphorylation of HSPB1 is mainly regulated by the Her2-p38 MAPK pathway and is significantly correlated with Her2 and lymph-node positivity[10,28].

Overexpression of Voltage Dependent Anion-selective Channel (VDAC1) was observed in the IDCA breast cancer cell lines studied. Shoshan-Barmatz and Mizrachi[31], Zhang *et al.*[28] and Shinohara *et al.*[32] have also reported the overexpression of VDAC1 in IDCA. The overexpression of VDAC1 could be linked to a number of apoptotic stimuli in the IDCA cell lines. The VDAC is located in the mitochondrial outer membrane, functions as gatekeeper for the entry and exit of mitochondrial metabolites and thus controls cross-talk between mitochondria and the cytosol[31,33].

The VDAC also serves as a site for the docking of cytosolic proteins, such as hexokinase and is recognized as a key protein in mitochondria-mediated apoptosis[31,32]. The role of VDAC in apoptosis has emerged from various studies showing its involvement in cytochrome C release and apoptotic cell death as well as its interaction with proteins regulating apoptosis, including the mitochondria-bound isoforms of hexokinase HK-I and HK-II[31,32].

The role of VDAC1 in regulating apoptosis has been the subject of considerable debate[31]. Knockout of all three isoforms of VDAC was shown to have no effect on mitochondrial apoptosis in mouse embryonic fibrosis[31,34], whereas conflicting data has indicated that the N-terminal of VDAC1 is essential for release of cytochrome C following various apoptotic stimuli[31,35]. The VDAC1 promotes aerobic glycolysis in cell lines through its interaction with hexokinase at the outer mitochondrial membrane[31,36,37].

Other studies have shown that hexokinase binds to mitochondria via the N-term of VDAC1 and that this is associated with resistance to mitochondrial apoptosis[31,35,38]. These reported interactions between VDAC1 and hexokinase imply a pro-survival rather than pro-apoptotic role for VDAC1 activity in cancerous cells.

Thus, VDAC may act as a Mitochondrial Permeability Transition Pore (MPTP) on the outer mitochondrial membrane. It was reported that O_2 but not H_2O_2 induces a rapid and massive release of cytochrome C from mitochondria, which is a central event in apoptosis[31,39,40]. Reduced cytotoxicity of FNQ13 in VDAC knockdown cells might be due to lower cytochrome C release and therefore, reduced induction of apoptosis[31,41].

On the other hand, VDAC1 in the plasma membrane has recently been shown to possess NADH-ferricyanide reductase activity, which could directly catalyze the reduction of ferricyanide in the presence of NADH[31,39]. Simamura *et al.*[41] showed that FNQs induces the NADH-dependent production of ROS on the mitochondrial outer membrane and proposes

that VDAC has a similar function as NAD(P)H-quinone oxidoreducase 1 and therefore, mitochondrial VDAC may catalyze the reduction of anticancer drugs such as furanonaphthoquinones and leading to mitochondrial production of ROS. However, NAD(P)H-quinone oxidoreductase 1, which activates mitomycin C is localized in the cytosol but not on the mitochondrial outer membrane and mediates the two-electron reduction of substrates, suggesting that VDAC and NAD(P)H quinone oxidoreductase[1] have different functions[31,42].

CONCLUSION

The combination of 2D-DIGE and nano-LC-MS/MS technologies enabled the identification of proteins differentially expressed between invasive ductal carcinoma (IDCA) and Ductal Carcinoma *In Situ* (DCIS) breast cancer cell lines. The expression levels of 60 kDa mitochondrial heat shock protein (HSPD1), Voltage Dependent Anion-selective Channel protein 1 (VDAC1) and Heat Shock Protein Beta 1 (HSPB1) were higher in IDCA. However, no far reaching conclusions can be made from the data obtained since cell lines were used in the study. Nevertheless, we were encouraged because other studies have identified differential expression of similar proteins in cancer. It is expected that this study will stimulate further studies utilizing tumor biopsies to determine if the 60 kDa HSPD1, VDAC1 and HSPB1 are also differentially expressed in clinical specimens. If independently validated using tumor biopsies, then HSPD1, VDAC1 and HSPB1 may represent a new set of biomarkers that can be used to predict the invasive potential of breast tumors.

ACKNOWLEDGMENTS

The authors wish to thank the Tertiary Education Trust Fund (TETF), Nigeria for their financial support of the study. The authors are grateful to Stephen Russell, Florentina Mayko and Steve Wolve of ITSI Biosciences LLC, Jownstown, PA, USA for their technical support. The cell lines used for the study were provided by George Iida, PhD, Windber Research Institute, Windber, PA, USA.

REFERENCES

1. Alabi, M.A., 2014. Proteins expression in pre-invasive and invasive breast carcinoma. Ph.D. Thesis, Biochemistry Department, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.
2. Jedy-Agba, E.E., M.P. Curado, E. Oga, M.O. Samaila and E.R. Ezeome *et al*., 2012. The role of hospital-based cancer registries in low and middle income countries-The Nigerian case study. Cancer Epiemiol., 36: 430-435.
3. ACS., 2015. Cancer facts and figures 2015. American Cancer Society, Atlanta, GA., USA., pp: 1-156. http://www.cancer.org/acs/groups/content/@editorial/documents/document/acspc-044552.pdf
4. Michaelson, J., S. Satija, R. Moore, G. Weber, E. Halpern, A. Garland and D.B. Kopans, 2001. Observations on invasive breast cancer diagnosed in a service screening and diagnostic breast imaging program. J. Women's Imaging, 3: 99-104.
5. Burstein, H.J., J.R. Harris and M. Morrow, 2008. Malignant Tumors of the Breast. In: DeVita, Hellman and Rosenberg's Cancer: Principles and Practice of Oncology, DeVita, V.T., T.S. Lawrence and S.A. Rosenberg (Eds.). Lippincott Williams and Wilkins, Philadelphia PA., USA., ISBN-13: 9780781772075, pp: 1606-1654.
6. Boyiri, T., R.I. Somiari, S. Russell, C. Aliaga and K. El-Bayoumy, 2009. Proteomics of rat prostate lobes treated with 2-N-hydroxylamino-1-methyl-6-phenylimidazo[4,5-b]pyridine, 5α-dihydrotestosterone, individually and in combination. Int. J. Oncol., 35: 559-567.
7. Somiari, R.I., A. Sullivan, S. Russell, S. Somiari and H. Hu *et al*., 2003. High-throughput proteomic analysis of human infiltrating ductal carcinoma of the breast. Proteomics, 3: 1863-1873.
8. Arciero, C., S.B. Somiari, C.D. Shriver, H. Brzeski and R. Jordan *et al*., 2003. Functional relationship and gene ontology classification of breast cancer biomarkers. Int. J. Biol. Markers, 18: 241-272.
9. Somiari, R.I., S. Somiari, S. Russell and C.D. Shriver, 2005. Proteomics of breast carcinoma. J. Chromatogr. B, 815: 215-225.
10. Kim, L.S. and J.H. Kim, 2011. Heat shock protein as molecular targets for breast cancer therapeutics. J. Breast Cancer, 14: 167-174.
11. Santagata, S., R. Hu, N.U. Lin, M.L. Mendillo and L.C. Collins *et al*., 2011. High levels of nuclear Heat-Shock Factor 1 (HSF1) are associated with poor prognosis in breast cancer. Proc. Natl. Acad. Sci. USA., 108: 18378-18383.
12. Gibert, B., B. Eckel, L. Fasquelle, M. Moulin and F. Bouhallier *et al*., 2012. Knock down of heat shock protein 27 (HSPB1) induces degradation of several putative client proteins. PLoS One, Vol. 7. 10.1371/journal.pone.0029719.
13. Sims, J.D., J. McCready and D.G. Jay, 2012. Extracellular Heat Shock Protein (HSP)70 and HSP90α assist in matrix metalloproteinase-2 activation and breast cancer cell migration and invasion. PLoS One, Vol. 64. 10.1371/journal.pone.0018848.
14. Ciocca, D.R. and S.K. Calderwood, 2005. Heat shock proteins in cancer: Diagnostic, prognostic, predictive and treatment implications. Cell Stress Chaperones, 10: 86-103.

15. Paepe, B.D., 2012. Mitochondrial markers for cancer: Relevance to diagnosis, therapy and prognosis and general understanding of malignant disease mechanisms. ISRN Pathol. 10.5402/2012/217162.
16. Romanucci, M., T. Bastow and L.D. Salda, 2008. Heat shock proteins in animal neoplasms and human tumours-a comparison. Cell Stress Chaperones, 133: 253-262.
17. Cappello, F., E.C. de Macario, L. Marasa, G. Zummo and A.J.L. Macario, 2008. Hsp60 expression, new locations, functions and perspectives for cancer diagnosis and therapy. Cancer Biol. Ther., 7: 801-809.
18. Cappello, F., M. Bellafiore, A. Palma, S. David and V. Marciano *et al*., 2003. 60KDa chaperonin (HSP60) is over-expressed during colorectal carcinogenesis. Eur. J. Histochem., 47: 105-110.
19. Kang, Y., W. He, S. Tulley, G.P. Gupta and I. Serganova *et al*., 2005. Breast cancer bone metastasis mediated by the smad tumor suppressor pathway. Proc. Natl. Acad. Sci. USA., 102: 13909-13914.
20. Lebret, T., R.W.G. Watson, V. Molinie, A. O'Neill, C. Gabriel, J.M. Fitzpatrick and H. Botto, 2003. Heat shock proteins HSP27, HSP60, HSP70 and HSP90: Expression in bladder carcinoma. Cancer, 98: 970-977.
21. Jaattela, M., 2002. Programmed cell death: Many ways for cells to die decently. Ann. Med., 346: 480-488.
22. Jolly, C. and R.I. Morimoto, 2000. Role of the heat shock response and molecular chaperones in oncogenesis and cell death. J. Natl. Cancer Inst., 92: 1564-1572.
23. Thanner, F., M.W. Sutterlin, M. Kapp, L. Rieger and A.K. Morr *et al*., 2005. Heat shock protein 27 is associated with decreased survival in node-negative breast cancer patients. Anticancer Res., 25: 1649-1653.
24. Mosser, D.D. and R.I. Morimoto, 2004. Molecular chaperones and the stress of oncogenesis. Oncogene, 23: 2907-2918.
25. Li, D.Q., L. Wang, F. Fei, Y.F. Hou and J.M. Luo *et al*., 2006. Identification of breast cancer metastasis-associated proteins in an isogenic tumor metastasis model using two-dimensional gel electrophoresis and liquid chromatography-ion trap-mass spectrometry. Proteomics, 6: 3352-3368.
26. Lemieux, P., S. Oesterreich, J.A. Lawrence, P.S. Steeg, S.G. Hilsenbeck, J.M. Harvey and S.A. Fuqua, 1997. The small heat shock protein hsp27 increases invasiveness but decreases motility of breast cancer cells. Invasion Metastasis, 17: 113-123.
27. Cayado-Gutierrez, N., V.L. Moncalero, E.M. Rosales, W. Beron and E.E. Salvatierra *et al*., 2013. Downregulation of HSP27 (HSPB1) in MCF-7 human breast cancer cells induces upregulation of PTEN. Cell Stress Chaperones, 18: 234-249.
28. Zhang, Y. and X. Shen, 2007. Heat shock protein 27 protects L929 cells from cisplatin-induced apoptosis by enhancing Akt activation and abating suppression of thioredoxin reductase activity. Clin. Cancer Res., 13: 2855-2864.
29. Rocchi, P., E. Beraldi, S. Ettinger, L. Fazli, R.L. Vessella, C. Nelson and M. Gleave, 2005. Increased HSP27 after androgen ablation facilitates androgen-independent progression in prostate cancer via signal transducers and activators of transcription 3-mediated suppression of apoptosis. Cancer Res., 65: 11083-11093.
30. Xu, J., L. Liao, G. Ning, H. Yoshida-Komiya, C. Deng and B.W. O'Malley, 2000. The steroid receptor coactivator SRC-3 (p/CIP/RAC3/AIB1/ACTR/TRAM-1) is required for normal growth, puberty, female reproductive function and mammary gland development. Proc. Natl. Acad. Sci. USA., 97: 6379-6384.
31. Shoshan-Barmatz, V. and D. Mizrachi, 2012. VDAC1: From structure to cancer therapy. Mol. Cell. Oncol., Vol. 2. 10.3389/fonc.2012.00164
32. Shinohara, Y., T. Ishida, M. Hino, N. Yamazaki, Y. Baba and H. Terada, 2000. Characterization of porin isoforms expressed in tumor cells. Eur. J. Biochem., 267: 6067-6073.
33. Chacko, A.D., F. Liberante, I. Paul, D.B. Longley and D.A. Fennell, 2010. Voltage dependent anion channel-1 regulates death receptor mediated apoptosis by enabling cleavage of caspase-8. BMC Cancer, Vol. 10. 10.1186/1471-2407-10-380
34. Baines, C.P., R.A. Kaiser, T. Sheiko, W.J. Craigen and J.D. Molkentin, 2007. Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. Nat. Cell Biol., 9: 550-555.
35. Abu-Hamad, S., N. Arbel, D. Calo, L. Arzoine and A. Israelson *et al*., 2009. The VDAC1 N-terminus is essential both for apoptosis and the protective effect of anti-apoptotic proteins. J. Cell Sci., 122: 1906-1916.
36. Majewski, N., V. Nogueira, P. Bhaskar, P.E. Coy and J.E. Skeen *et al*., 2004. Hexokinase-mitochondria interaction mediated by akt is required to inhibit apoptosis in the presence or absence of bax and bak. Mol. Cell, 16: 819-830.
37. Pedersen, P.L., 2008. Voltage Dependent Anion Channels (VDACs): A brief introduction with a focus on the outer mitochondrial compartment's roles together with hexokinase-2 in the Warburg effect in cancer. J. Bioenergetics Biomembr., 40: 123-126.
38. Pastorino, J.G., N. Shulga and J.B. Hoek, 2002. Mitochondrial binding of hexokinase II inhibits bax-induced cytochrome c release and apoptosis. J. Biol. Chem., 277: 7610-7618.
39. Baker, M.A., D.J.R. Lane, J.D. Ly, D. de Pinto and A. Lawen, 2004. VDAC1 is a transplasma membrane NADH-ferricyanide reductase. J. Biol. Chem., 279: 4811-4819.

40. Madesh, M. and G. Hajnoczky, 2001. VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome c release. J. Cell Biol., 155: 1003-1016.
41. Simamura, E., K.I. Hirai, H. Shimada, J. Koyama, Y. Niwa and S. Shimizu, 2006. Furanonaphthoquinones cause apoptosis of cancer cells by inducing the production of reactive oxygen species by the mitochondrial voltage-dependent anion channel. Cancer Biol. Ther., 5: 1523-1529.
42. Okada, S.F., W.K. O'Neal, P. Huang, R.A. Nicholas and L.E. Ostrowski *et al.*, 2004. Voltage-Dependent Anion Channel-1 (VDAC-1) contributes to ATP release and cell volume regulation in murine cells. J. Gen. Physiol., 124: 513-526.