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Novel predication of protein biomarkers in interferon-gamma-stimulated breast cancer cells

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ABSTRACT

Objective: Proteomics is the large-scale study of localization, identification, structure, and function of the proteome. A proteome is the complete set of proteins expressed and modified by an organism under a specific set of environmental conditions. This study was undertaken to investigate the novel protein biomarkers that play a role in breast cancer under inflammatory condition.

Methods: The two-dimensional gel electrophoresis (2-DE) was applied in the context of the breast cancer model system to investigate the effect of interferon-gamma (IFN-γ) on the differential protein expression in breast cancer-derived cell lines CAMA-1 and 3,4-methylenedioxyamphetamine (MDA)-MB-231. Whole cell lysates were prepared from IFN-γ-stimulated and non-stimulated CAMA-1 and MDA-MB-231 cells for 2-DE to obtain information for potential differential protein expression. Protein spots in the gels were visualized by silver staining and analyzed by Progenesis SameSpot. Gels were then scanned using the Epson image scanner with LabScan 6.0 software. The ExPASy tool was used to identify and quantify breast cancer cell membrane proteins expressed in response to IFN-γ.

Results: In the present proteomics study, a series of differentially expressed proteins were analyzed in IFN-γ-stimulated CAMA-1 and MDA-MB-231 cells. While results obtained from this analysis can be used as preliminary data to identify differences between untreated and IFN-γ-treated samples, they were not used for further mass spectrometry analysis.

Conclusion: The data described and discussed here can be utilized for further data validation projects and could assist in the discovery of new breast cancer-related proteins and molecular pathways.

Keywords: Breast cancer cells, interferon-gamma, protein spot, proteomics, two-dimensional gel electrophoresis

Introduction

Proteomics is the large-scale study of localization, identification, structure, and function of the proteome. A proteome is the complete set of proteins expressed and modified by an organism under a specific set of environmental conditions. The application of mass spectrometry (MS) in proteomics analysis has made it a powerful tool for protein characterization [1] The classical method for the quantitative analysis of complex protein mixtures is the separation of proteins by two-dimensional gel electrophoresis (2-DE) and identification of resolved proteins by MS or tandem MS (MS/MS).[2] 2-DE accommodates a large mass range and permits the analysis of the entire set of proteins. Furthermore, proteins are separated with high resolution by isoelectric point (pI) and molecular mass. Resolved protein spots are used for comparison among different samples and can be used for MS analysis. However, this method has some disadvantages such as the occurrence of gel to gel variation, a limited dynamic range, and difficulty in detection of basic or hydrophobic proteins and low range of molecular weights and limited range pIs of proteins.[3] Recent developments of non-gel-based and label-free shotgun proteomics techniques have rendered quantitative protein study faster, cleaner, and simpler.[4-6] Proteomics studies provide the global analysis of protein expression and function. In contrast to the genome, the proteome is very dynamic in nature due to post-translational modifications.[7,8] Therefore, to recognize the physiological and pathological events that occur in health and disease, it is important to detect and analyze the proteins from their native proteome. Interferon-gamma (IFN-γ) has now well considered as a pleiotropic cytokine played a major role as an effector molecule for antitumor immunity which has ability

to suppress tumor growth. On the other hand, it is also known as a tumor promoter involved in promoting an outgrowth of tumor cells.[9,10] Not only in cancer but this pleiotropic cytokine is also involved in various other mechanisms.[11] Taking into consideration the importance of proteomics, 2-DE was applied in the context of the breast cancer model system to investigate the effect of cytokines such as IFN-γ on breast cancer cells. The ExPASy tool (http://web.expasy.org/tagident/) was used to identify and quantify breast cancer cell membrane proteins expressed in response to IFN-γ. This investigation is a potential aid in developing an understanding of the molecular aspects of breast cancer and also helpful for the drug designing for cancer patients.

Methods

Place of work

This work was conducted in College of Medicine, Qassim University, Buraidah, KSA, on breast cancer cell lines CAMA-1 and 3,4-methylenedioxyamphetamine (MDA)-MB-231 which were commercially purchased from American Type Culture Collection (ATCC, Breast Cancer Cell Panel # ATCC® 30-4500K™, Middlesex, UK).

Treatment of CAMA-1 and MDA-MB-231 with interferon-gamma (IFN-γ)

The human mammary gland cell lines, CAMA-1 and MDA-MB-231, were treated with INF-γ (Sigma-Aldrich, St-Louise, USA) as described previously.[12,13]

Preparation of cell lysate

The cell lysate was prepared as described previously.[14,15] The untreated and IFN-γ-treated CAMA-1 and MDA-MB-231 cells in 100 mm cell culture dishes (Nunc, UK), with density of 1×10^7 cells were used per sample to make cell lysate. The cells were detached from culture dishes using Accutase, counted, and washed with phosphate-buffered saline. The cell pellet was resuspended in 1 ml of the protein extraction buffer containing 7 M urea, 2 M thiourea, 4% CHAPS, ×1 protease inhibitor cocktail, 20 mM DTT, 1% ampholyte, and benzonase. Cells were vortexed and sonicated for 5 min at 4°C. Cell lysates were spun at 20,000 rpm for 10 min; supernatant was collected into a clean microcentrifuge tube and stored at −80°C for later use. The protein concentration of cell lysates was estimated using the Coo Assay (Uptima, Interchim, France) following manufacturer's instructions.

First-dimension gel isoelectric focusing (IEF)

The first-dimension gel IEF was performed by Immobiline DryStrip gels as per instructions of the manufacturer (GE Healthcare Life Sciences, Amersham Place.

Little Chalfont, Buckinghamshire, HP7 9NA UK). Briefly, the Immobiline DryStrip gel pH 3–10 NL, 18 cm used in this study was rehydrated in an Immobiline DryStrip reswelling tray. The dry strip was rehydrated for 24 h at room temperature in 350 µl of rehydration buffer (7M urea, 2M thiourea, 4% CHAPS, ×1 protease inhibitor cocktail, 20 mM DTT, 1% ampholyte, 0.1% bromophenol blue, and 0.05% sodium dodecyl sulfate [SDS]) containing 80 µg of sample proteins. The immobilized pH gradient (IPG) strip was overlaid using approximately 3 ml of Immobiline PlusOne DryStrip cover fluid (GE Healthcare Bio-Sciences AB). After rehydration, the IPG strip was briefly rinsed with ultrapure water to remove crystallized urea.

For IEF, the IPGPhor was cleaned with strip holder cleaning solution (GE Healthcare Bio-Sciences AB). The Ettan IPGPhor3 was switched on and connection with the IPGPhor3 control software was established. IPGPhor manifold was covered with 108 ml of Immobiline PlusOne DryStrip cover fluid and the rehydrated strips were then placed in individual lanes of Ettan IPG strip holder (GE Healthcare Bio-Sciences AB) under the fluid using tweezers with the positive end toward the anode end of the manifold. Hydrated filter wicks were placed between the IPG strips and the electrodes. The cathodic (-ve) filter wick was rehydrated with 150 µL of 100 mM DTT. The anodic (+ve) filter wick was hydrated with 150 µL miliQ water. The lid was closed and IPGPhor program was run according to the programme below. A holding step at the end was added to so that it could be left overnight.

Step 1	200 V	500 V h
Step 2	500 V	500 V h
Grad 3	1000 V	800 V h
Grad 4	10,000 V	16,500 V h
Step 5	10,000 V	6200 V h
Step 6	200 V	24 h

At the end of the program, the computer was disconnected and IPGPhor was stopped. The paper wicks were removed with tweezers and discarded. The IPG strips were placed in a Petri dish, rinsed briefly with deionized water, labeled, and stored at −80°C for later use.

Second-dimension gel electrophoresis

The second-dimension gel electrophoresis (2D gel electrophoresis) was performed by BIO-RAD PROTEAN® II Assembly as per instructions given by the manufacturer (Bio-Rad Laboratories Ltd., Watford Hertfordshire, WD17, 1ET). Briefly, the glass plates were cleaned with 70% ethanol, dried, assembled with 2 mm spacers, and clipped into the casting frame. Purite water was poured between the plates to check for leakage. Assemblies that leaked were taken apart and reclipped and the process was repeated. On establishing a non-leaking system, the water was removed and the system was dried *in situ* with pressurized airflow. The gel solution 12% SDS polyacrylamide gel electrophoresis (SDS-PAGE) was made with water, 1.5 M Tris-HCl, and 30% acrylamide solution. These were placed in a flask and degassed for 15 min at ambient temperature. The tetramethylethylenediamine,

ammonium persulfate (APS), and SDS were added and mixed by stirring. The gel solution was poured in between glass plates, avoiding any air bubbles, to 1 cm below the lowest plate. The top of the gels was covered with overlay buffer (water saturated isopropanol 80%) and allowed to polymerize overnight. The 2 D electrophoresis was performed in the following steps.

Equilibration of the IPG strips

Before the 2D gel run, the IPG strips containing isoelectrically focused proteins were equilibrated and reduced. For each strip, two vials of 10 ml aliquots of the frozen equilibration buffer were thawed at room temperature. In one vial of equilibration buffer, 100 mg of DL-dithiothreitol (DTT) was added while in the other, 400 mg of iodoacetamide was added and allowed to mix gently. The IPG strips were first equilibrated in equilibration buffer containing 1% DTT and then in a buffer containing 4% iodoacetamide for 15 min each at room temperature. The IPG strips were rinsed with ×1 electrophoresis buffer before placing on the second-dimension gel.

Assembly and running of 2D gel

Agarose sealing solution was heated to liquefy. IPG strips were trimmed from each end up to 0.6 cm, thus giving a final length of 16 cm. A small square of paper electrode wick (2 × 3 cm half thickness) was loaded with 10 µl of molecular weight marker and placed on the top of the left-hand corner of the gel. The IPG strip was placed into the well of the 12% SDS-PAGE gel with the acidic side facing the glass plate hinge and sealed with agarose solution, avoiding any air bubbles. The electrophoresis tank was filled with 1.5 L of gel running buffer. The gels with strips were removed from the casting assembly and clipped onto the core unit of the protean tank. The core unit was lifted into the tank, running buffer was added to the top of the upper buffer chamber and air bubbles were removed with a glass rod. The lid was fitted to the tank and cables were connected to the power Pac (Bio-Rad, Power Pac 1000). Electrophoresis was carried out first at 50 V for 30 min and then at 150 V for about 4.5 h or until the bromophenol blue dye front had reached to the lower end. The core unit was then removed from the tank disassembled and gels were removed from the clamps. The spacers were loosened, and one edge of the glass plate was lifted up with a spatula. The gel was then placed in a glass container containing gel fixing solution.

Silver nitrate staining

Protein spots were visualized by silver nitrate staining as described previously.[16] Briefly, after electrophoresis gels were fixed for half an hour in fixing solution, they were sensitized for 30 min and washed with ultrapure water. Staining was carried out using 2.5% silver nitrate solution for 20 min followed by a careful wash with ultrapure water for a maximum of 1 min. The gels were developed for 10–15 min until spots appeared, and the reaction was stopped by washing with stop solution for 10 min. The gels were washed 3 times with ultrapure water and stored in gel preserving solution at 4°C.

Gel image capture and spot analysis

Gels were scanned using the scanner (Epson image scanner III) with LabScan 6.0 software. First, the scanner was calibrated and set to use the transparent settings at 300 dpi with the blue filter. The scanner surface was cleaned with 70% ethanol and a little purite water was poured on the surface. The gel was placed directly on the scanner, previewed and air bubbles were smoothed out if any were present. The scan area of the gel was then selected and scanned. Gel images were saved as mel and tiff files. Scanned gel images were characterized with Progenesis SameSpot software package (Nonlinear Dynamics Limited, UK).

Results

2D gel analysis of CAMA-1 and MDA-MB-231 cells

CAMA-1 and MDA-MB-231 cells were used in this study as a model for breast cancer and differential expression of the breast cancer proteome in response to pro-inflammatory cytokines such as IFN-γ. This was investigated by 2-DE followed by protein spot analysis. The total proteome is separated by the first-dimension isoelectric focusing on the basis of the isoelectric points (pI) of the various proteins. The isoelectrically focused proteins were resolved by the second-dimension SDS-PAGE, as shown in Figures 1-3. Silver-stained protein spots in untreated CAMA-1 and MDA-MB-231 cells and IFN-γ treated cells, as well as a third unchallenged control group, are shown in Figures 2a-d and 4a-d. Gel images from three independent experiments were then analyzed using Progenesis SameSpot software (Nonlinear Dynamics Limited), where a control untreated sample gel was used as a reference to identify differentially expressed proteins. Differentially expressed protein spots from CAMA-1 and MDA-MB-231 cells are shown in Figures 1 and 3 surrounded by a blue line and a spot number is given to each spot. Some selected differentially expressed spots are also indicated with red arrows in all four conditions: Control cells only; in 10% FCS medium, cells treated with 100 IU/ml IFN-γ, cells treated with 500 IU/ml IFN-γ, and cells treated with 1000 IU/ml IFN-γ [Figures 2a-d and 4a-d]. These data were used to gather preliminary results to discover the potential of differential protein expression in response to infections in the model system. An example of a 3D spot graph analyzed by Progenesis SameSpot software is shown in Figure 5. Table 1 represents a summary of data obtained from a reference image showing all differentially expressed spots on an untreated control of CAMA-1 and MDA-MB-231 cells by PI and molecular weight. Representation and comparison of the number of spots in the untreated control of CAMA-1 or MDA-MB-231 and IFN-γ-treated CAMA-1 or MDA-MB-231 cells were identified by Progenesis SameSpot software and are shown in Tables 2 and 3.

Discussion

A proteomics study using 2-DE was performed to identify differences between untreated and IFN-γ-treated CAMA-1 and MDA-MB-231 cells. In comparison with IFN-γ-stimulated and non-stimulated CAMA-1 and MDA-MB-231 cells, 19 protein

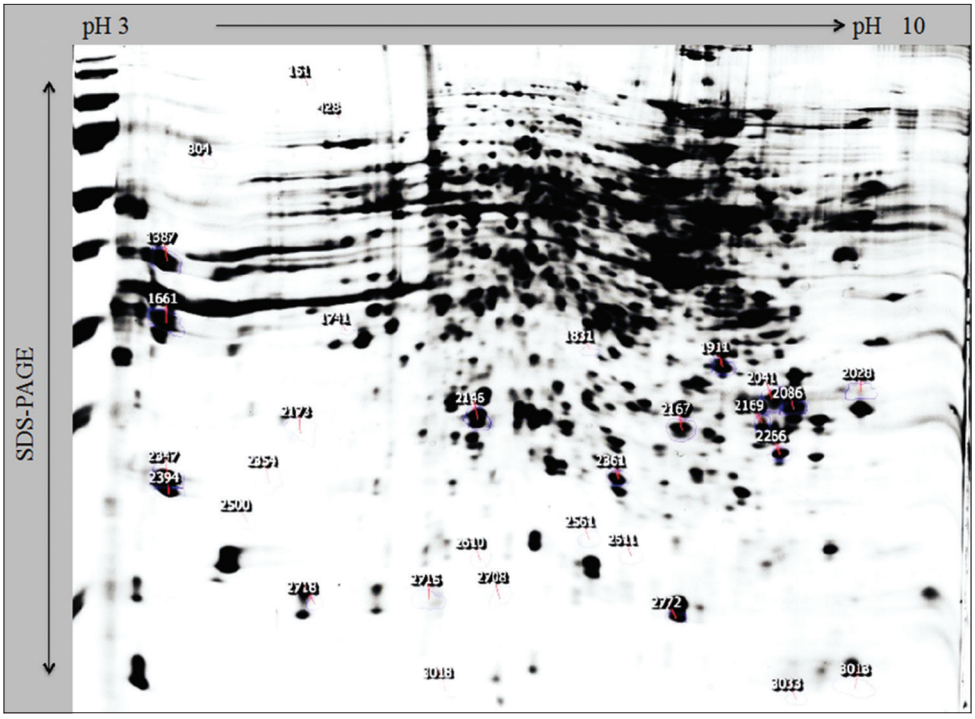

Figure 1: Representation of reference image showing an example image of spot detection on untreated CAMA-1 cells control Reference image used for image analysis that was carried out with Progenesis SameSpot software. Isoelectric point pI range is shown at the top, while the direction of the second-dimension SDS-PAGE is represented by an arrow on the left

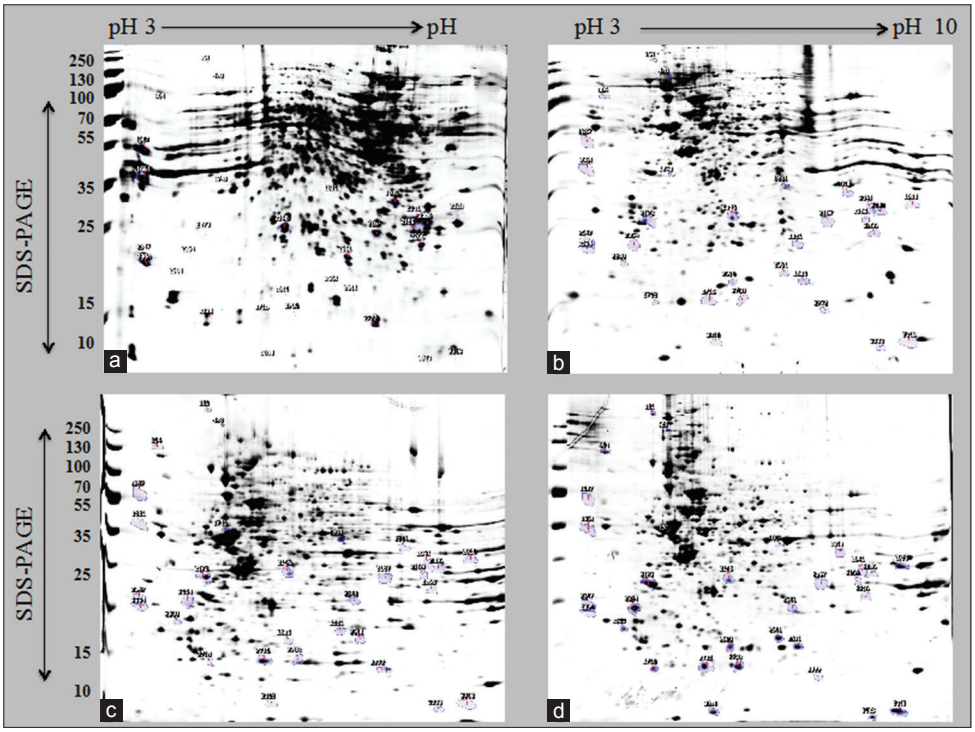

Figure 2: Representative two-dimensional gel images of total cell proteins from CAMA-1 cells untreated and treated with interferon-gamma (IFN-γ). (a) Control (untreated) cells only in 10% FCS medium. (b) Cells treated with 100 IU/ml IFN-γ. (c) Cells treated with 500 IU/ml IFN-γ. (d) Cells treated with 1000 IU/ml IFN-γ. All incubations were for 72 h. In the first dimension, 80 µg total soluble protein was separated on Immobiline IPG strips (18 cm, pH 3–10 NL). Isoelectric focusing was performed using an IPGPhor unit. The second dimension was performed on 12% SDS-PAGE gels, with image analysis carried out using Progenesis SameSpot software. M - Molecular mass standards are shown on the left side (kDa - kiloDaltons). Differentially expressed, user selected protein spots in four gels are marked by red arrows

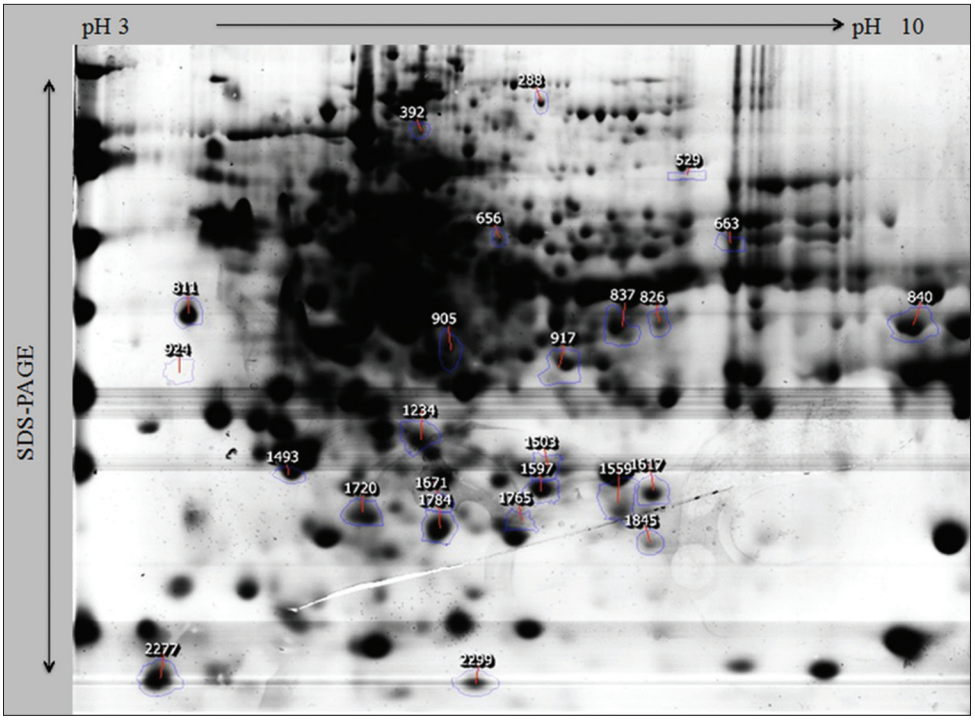

Figure 3: Representation of reference image showing an example image of spot detection on untreated of 3,4-methylenedioxyamphetamine-MB-231 cells control. Reference image used for image analysis that was carried out with Progenesis SameSpot software. Isoelectric point pI range is shown at the top, while the direction of the second-dimension SDS-PAGE is represented by an arrow on the left

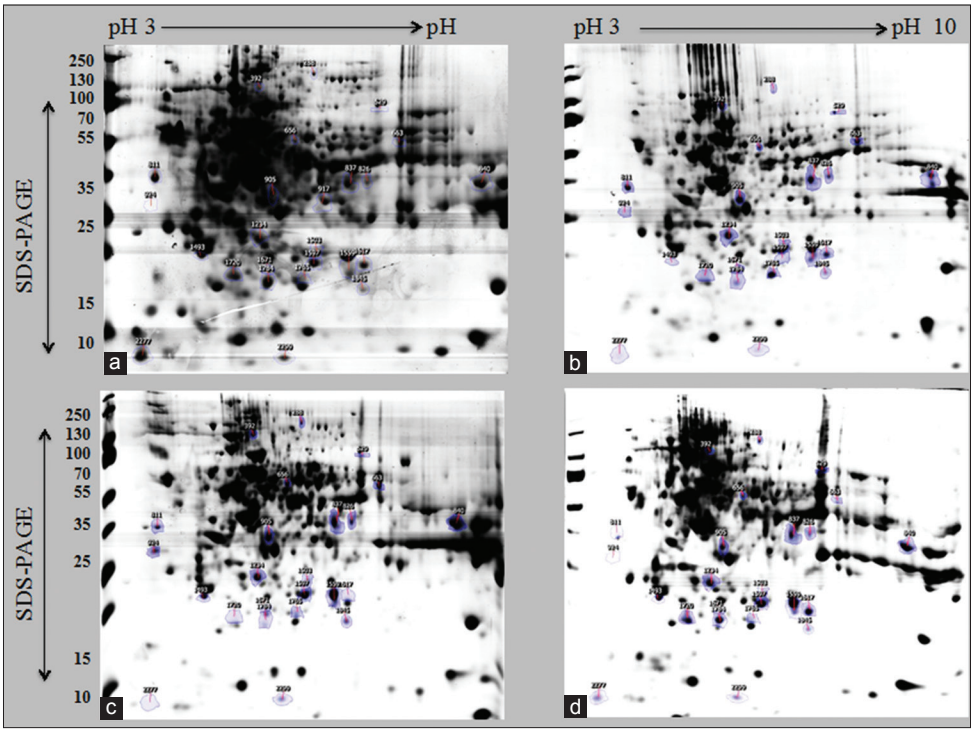

Figure 4: Representative two-dimensional gel images of 3,4-methylenedioxyamphetamine-MB-231 total cell proteins from cells untreated and treated with interferon-gamma (IFN-γ) (a) Control (untreated) cells only in 10% FCS medium. (b) Cells treated with 100 IU/ml IFN-γ. (c) Cells treated with 500 IU/ml IFN-γ. (d) Cells treated with 1000 IU/ml IFN-γ. Incubations were for 72 h. In the first dimension, 80 µg total soluble protein was separated on Immobiline IPG strips (18 cm, pH 3–10 NL). Isoelectric focusing was performed on an IPGPhor unit. The second dimension was performed on 12% SDS-PAGE gels, with image analysis carried out with Progenesis SameSpot software. M - Molecular mass standards are shown on the left side (kDa - kiloDaltons). Differentially expressed, user selected protein spots in four gels are marked by red arrows

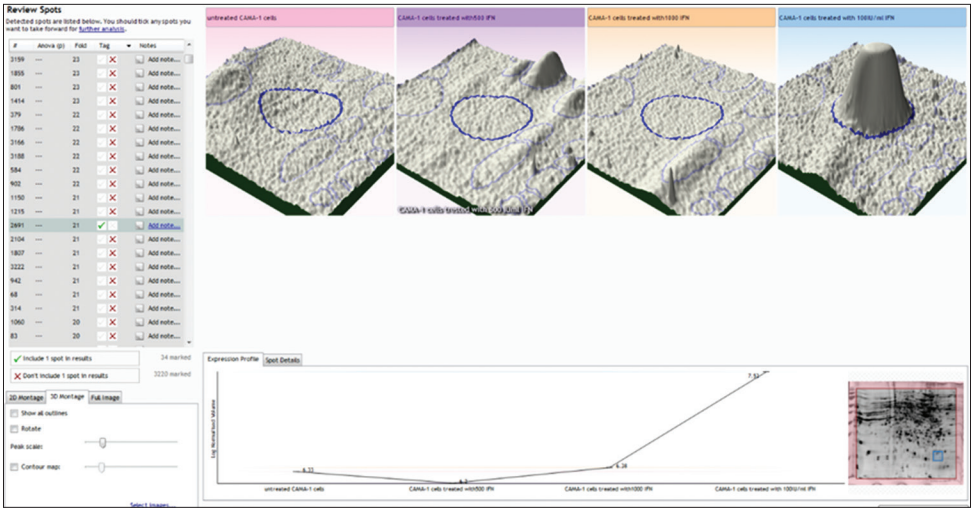

Figure 5: Example of three-dimensional (3D) spot graph analyzed by Progenesis SameSpot software. 3D spot graph showing the difference between untreated (control) and treated CAMA-1 cells treated with different doses of interferon-gamma (IFN-γ). The images show the effect of IFN-γ on breast cancer cells

Table 1: Summary of data obtained from reference image showing all differentially expressed spots on untreated CAMA-1 and MDA-MB-231 cells controls by pI and molecular weight

CAMA-1 cells			MDA-MB-231 cells		
Spot number	PI (pH)	Molecular weight (kDa)	Spot number	PI (pH)	Molecular weight (kDa)
2610	6	16	1720	4	20
2708	7	12	1765	6	18
2561	7	18	1597	7	18
2611	8	17	1671	5	35
2715	7	16	2277	3	10
3018	7	12	2299	5	10
3033	8	11	1784	5	22
2718	5	15	1845	8	22
3013	10	12	1493	3	30
2500	4	18	663	9	65
2086	9	25	837	6	60
2167	8	22	392	4	110
1387	3	30	656	6	63
1741	6	25	1234	4	35
2266	9	20	1503	7	20
2361	7	20	826	7	60
2410	8	18	905	5	55
2347	3	20			
2354	5	22			

MDA: 3,4-Methylenedioxyamphetamine

spots resolved at a given pI and molecular weight were identified in CAMA-1 cells and 17 spots were identified in MDA-MB-231 cells. The 2-DE results were analyzed using ExPASy tool that yielded several potential useful results. The polypeptide spots that randomly selected and analyzed were highlighted as shown in Table 1-3. Five spots of the 19 polypeptide spots generated from the CAMA-1 cell sample were randomly selected and analyzed. For example, the polypeptide spot number 1741 correlated with IFN-induced transmembrane protein 10, which has a pI of ~ 6 and an MW 25 kDa. The polypeptide spot number 2610, which it is believed to be related to programmed cell death protein 5 (PDCD5), was also evaluated. PDCD5 has a pI of ~ 6 and an MW 16 kDa which is very similar to spot number 2610. PDCD5 is widely expressed in most types of normal human tissue and is unregulated in cells undergoing apoptosis.[17] It has since been confirmed that IFN-γ inhibits

Table 2: Representation and comparison of the number of spots in untreated CAMA-1 cells control and in IFN-γ treated CAMA-1 cells, identified by progenesis samespot software

Average normalized volumes of CAMA-1 cells spot				
Spot number	**Untreated CAMA-1 (control)**	**CAMA-1 treated with 100 IU/ml of IFN-γ**	**CAMA-1 treated with 500 IU/ml of IFN-γ**	**CAMA-1 treated with 1000 IU/ml of IFN-γ**
2610	Spot-	Spot-	Spot+	Spot-
2708	Spot-	Spot-	Spot+	Spot-
2561	Spot-	Spot-	Spot+	Spot-
2611	Spot-	Spot-	Spot+	Spot-
2715	Spot-	Spot++	Spot+	Spot-
3018	Spot-	Spot-	Spot+	Spot-
3033	Spot-	Spot-	Spot+	Spot-
2718	Spot-	Spot-	Spot+	Spot-
3013	Spot-	Spot-	Spot+	Spot-
2500	Spot-	Spot-	Spot+	Spot-
2086	Spot+	Spot-	Spot-	Spot-
2167	Spot+	Spot-	Spot-	Spot-
1387	Spot+	Spot-	Spot-	Spot-
1741	Spot-	Spot+	Spot+	Spot-
2266	Spot+	Spot-	Spot-	Spot-
2361	Spot+	Spot-	Spot-	Spot-
2410	Spot+	Spot-	Spot-	Spot-
2347	Spot+	Spot-	Spot-	Spot-
2354	Spot-	Spot-	Spot+	Spot-

CAMA-1 cells were either untreated or treated with the indicated concentration of IFN-γ for a period of 72 h. Both untreated and treated cell lysates were then subjected to isoelectric focusing. The second dimension was resolved with 12% SDS-PAGE. A plus sign (+) indicates the presence of spots or increase of spot size before or after treatment with IFN-γ. A minus sign (-) indicates the absence of spots before or after treatment with IFN-γ. Five spots of the 19 polypeptide spots (highlighted in bold) generated from the CAMA-1 cell sample were randomly selected and analyzed using ExPASy tool (http://web.expasy.org/tagident/). MDA: 3,4-Methylenedioxyamphetamine, IFN-γ: Interferon-gamma, SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

growth of human carcinoma cells through caspase-1-dependent induction of apoptosis.[18] It was assumed that IFN-γ played a critical role in the apoptosis-inducing factors like PDCD5. The investigation of spot number 1387 which has a pI of ~ 3 and an MW 30 kDa showed a strong similarity to cell cycle checkpoint protein RAD1. Cycle checkpoint proteins play an important role in controlling cell division of damaged cells.[19] Another interesting polypeptide spot number was 2086 which has a pI of ~ 9 and an MW 25 kDa. The search engine showed that this spot might be related TNF-α-induced protein 8-like protein 2 (TIPE2). TIPE2 is a novel immune negative molecule and an inhibitor of the oncogenic Ras in mice. However, its function in humans is still unclear.[20] The polypeptide spot number 2361 which has a pI of ~ 7 and an MW 20 kDa showed correlation with cyclin-dependent kinase 4. Cyclin-dependent kinases (CDKs) play a central role in the orderly transition from one phase of the eukaryotic mitotic cell division cycle to the next.[21,22] CDKs regulate cell proliferation and coordinate the cell cycle checkpoint response to DNA damage. Due to this, it is assumed that the absence of spot number 2361 after IFN-γ treatment may act as a regulator of tumor cell division. CDK4/6 inhibitors have been proven to be attractive antineoplastic agents due to the importance of CDK4/6 activity in regulating cell proliferation.[23] In the above manner, five spots of 17, the polypeptide spots obtained from MDAMB-231 cell lysates were randomly selected and analyzed. The polypeptide spot number 932, which has a pI of ~ 4 and an MW 110 kDa, has been investigated. This spot showed a strong correlation with melanoma-associated antigen C. Melanoma-associated antigens (MAGEs) are classified into two subgroups, I and II. Subgroup I consists of antigens in which expression is generally restricted to tumor or germ cells, whereas Subgroup II MAGEs are expressed in various normal adult human tissues.[24] Another polypeptide spot that was linked to ExPASy tool was number 905, which has a pI of ~ 5 and an MW 55 kDa. The database showed that spot number 905 corresponds to Myc proto-oncogene protein. The Myc proto-oncogene is a "master regulator" which controls many functions including cellular metabolism and proliferation. The Myc oncogene has been shown to induce apoptosis and has, therefore, been targeted to develop novel cancer therapies.[18] It was noticed that the data obtained from 2-DE indicate that the intensity of spot number 905 increased after IFN-γ treatment. In this context, IFN-γ has been shown to induce apoptosis of human carcinoma cells through a caspase-1-dependent mechanism, which is believed to control Myc proto-oncogene.[25] Therefore, IFN-γ might increase the apoptosis of breast cancer cells through Myc proto-oncogene protein in the same manner. TP53-regulated inhibitor of apoptosis is linked to the polypeptide spot number 2299, which has a pI of ~ 5 and an MW 10 kDa. The polypeptide spot number 1720, which has a pI of ~ 4 and an MW 20 kDa, showed correlation with growth arrest and DNA damage-inducible protein (GADD45β). GADD45β has

Table 3: Representation and comparison of the number of spots in untreated MDA-MB-21 cells control and in IFN-γ-treated MDA-MB-231 cells, identified by progenesis samespot software

Average normalized volumes of MDA-MB-231 cells spots				
Spot number	**Untreated MDA-MB-231 (control)**	**MDA-MB-231 treated with 100 IU/ml of IFN-γ**	**MDA-MB-231 treated with 500 IU/ml of IFN-γ**	**MDA-MB-231 treated with 1000 IU/ml of IFN-γ**
1720	Spot++	Spot+	Spot-	Spot-
1765	Spot-	Spot-	Spot-	Spot+
1597	Spot+	Spot+	Spot+	Spot-
1671	Spot-	Spot-	Spot-	Spot+
2277	Spot++	Spot+	Spot-	Spot-
2299	Spot++	Spot+	Spot+	Spot-
1784	Spot+++	Spot++	Spot+	Spot+
1845	Spot++++	Spot+++	Spot++	Spot+
1493	Spot++++	Spot+++	Spot++	Spot-
663	Spot++	Spot-	Spot+++	Spot+
837	Spot++	Spot+	Spot++	Spot+
932	Spot+	Spot+	Spot+	Spot+++
656	Spot-	Spot-	Spot+	Spot+
1234	Spot-	Spot++	Spot+++	Spot+++
1503	Spot+	Spot+	Spot+	Spot+
826	Spot+	Spot++	Spot+++	Spot+++
905	Spot+	Spot++	Spot+++	Spot+++

MDA-MB-231 cells were either untreated or treated with the indicated concentration of IFN-γ for a period of 72 h. Both untreated and treated cell lysates were then subjected to IEF isoelectric focusing. The second dimension was resolved with 12% SDS-PAGE. A plus sign (+) indicates the presence of spots or increase in spot size before or after treatment with IFN-γ. A minus sign (-) indicates the absence of spots before or after treatment with IFN-γ. Five spots of the 17 polypeptide spots (highlighted in bold) generated from the MDA-MB-231 cell sample were randomly selected and analyzed using ExPASy tool (http://web.expasy.org/tagident/). SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis, MDA: 3,4-Methylenedioxyamphetamine, IFN-γ: Interferon-gamma

been reported to inhibit apoptosis through attenuating c-Jun N-terminal kinase activation. It has been reported that TNF-α treatment induces GADD45β protein expression through nuclear factor-kappa B-mediated transcription, and transforming growth factor-b induces its expression.[26] The polypeptide spot number 1493 was then evaluated, which it is believed to be related to the HLA-DR alpha chain. The HLA-DR alpha chain has a pI of ~2 and MW 30 kDa, which is very similar to spot, number 1493. The expression of HLA-DR on cancer cells closely relates to a more favorable prognosis for cancer patients, but the immunological and non-immunological mechanisms are still obscure.[26]

Conclusion

The current study has found a number of differentially expressed proteins in the breast cancer model system in response to infectious agents. The data presented here could be used as baseline for further detailed investigation. In this regard, functional assays need to be employed to further validate the data presented. Further, analysis by mass spectrometry is also required to identify sequences and differentially observed polypeptide spots.

Declaration of Interest

The authors report no conflicts of interest.

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