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Proteins Which have been Found in Breast Cancer by Proteomic's Analyzer

Stephen J Petteruti*

Intellectual Medicine, Warwick RI, USA

*Corresponding author: Stephen J Petteruti, Intellectual Medicine, Warwick RI, USA

Abstract

Breast cancer is one of the most common types of invasive cancer in females worldwide. Despite major advances in early cancer detection and emerging therapeutic strategies, further improvement has to be achieved for precise diagnosis to reduce the chance of metastasis and relapses. Recently, proteomics based analyses of breast serum and tissue lysates have resulted in the finding of a number of potential tumor biomarkers providing, therefore, a basis for a better understanding of the breast-cancer development and progression, and eventually serving as diagnostic and prognostic markers. In this review, we examined the current Proteomics techniques applied to breast cancer studies and Proteins which have been found in this cancer.

Keywords: Proteins - breast cancer –proteomic's- analyzer

Introduction

The human genome contains approximately 35,000 genes and has the ability to encode up to 35,000 corresponding proteins. The occurrence of alternative RNA splicing and post-trans¬lational modifications (PTM), including phosphorylations, acetylations, glycosylations and protein cleavages may increase the expression of proteins to 500,000-1,000,000 [1]. Providing the direct link between gene sequence and cell physiology, proteomics is expected to complement genomic analyses to evaluate disease development, prognosis and response to treatment [2]. Worldwide, it is estimated that breast cancer is by far the most frequent cancer among women; each year, around 1.5 million new breast cancer cases are diagnosed in women throughout the world. Statistically, this means that 500,000 women worldwide will die from this disease [3]. In recent years breast cancer mortality rates have declined as a result of earlier detection, more effective therapies, mainly due to detection of breast cancer at earliest stages, might allow for more favorable results [4]. Therefore, there is an important need to improve the screening and diagnosis of early invasive and noninvasive tumors [5]. At present, finding novel, pre-symptomatic screening approaches are crucial in breast cancer screening and diagnosis, and have the potential to reduce mortality caused by this disease [6]. Identifying new protein markers in screening investigations can possibly avoid many deaths caused by this type of tumor [7]. Therefore, the search for specific disease-associated biomarker signatures is of particular interest since they could be applied in a standard clinical setting. Biomarker discovery for this disease is still very much in its discovery phase [8]. Multiple approaches have been developed that hold promise for the identification of serum biomarkers. Among them, quantitative proteomics yields information that specifically recognizes the differences between samples [9]. Numerous studies have already shown that this methodology can be used to uncover proteomic expression patterns linked with cancer, and some expression patterns have shown high promise to discover new biomarkers of early-stage cancers [10].

Recently, proteomics-based analyses of breast serum and tissue lysates have resulted in the finding of a number of potential tumor biomarkers providing, therefore, a basis for a better understanding of the breast-cancer development and progression, and eventually serving as diagnostic and prognostic markers [11]. In this review, the current

Proteomics techniques applied to breast cancer studies and Proteins which have been found in this cancer.

The proteomic tools for identifying molecular markers of the Breast

Different classifications of technologies for proteomic studies that are used for an analysis of tumor tissues and body fluids are known [12]. By the type of equipment used in the research, one may classify the proteomic technologies as follows: methods of gel electrophoresis (2D-PAGE, 2D-DIGE), peptide-oriented proteomics (LC combined with MS/MS: LC-MS/MS), the methods based on the use of arrays (RPPA) [13]. MS-based proteomic platforms for cancer studies and their principles of use are discussed in detail in [14]. To these platforms belong such methods as gel electrophoresis (1D-PAGE, 2D-PAGE (SDS-PAGE), 2D-DIGE), liquid chromatography (LC/MALDI or LC/MS (LC-MS/MS)), 2D-LC or multidimensional protein identification technology (MudiPuT), LC-ESI-MS, mass spectrometry (ion sources (ESI MS, MALDI MS, SELDI MS) combined with mass analyzers (Q MS, TOF MS, FT-ICR MS): MALDI-TOF MS, SELDI-TOF MS, ESI-MS/MS) [15]. By the data, LC-MS/MS is used mostly with bottom-up strategy, along with this some methodologies based on top-down strategy are already developed, too [16]. Also, for identification of new cancer biomarkers and potential therapeutic targets LC-MS/MS could be combined with quantitative methods: ICAT-LC-MS/MS, iTRAQ-LC-MS/MS, SILAC-LC-MS/MS [17].

In recent years, the combination of 2-DE and MS has been utilized extensively for proteomics research in medicine [18]. The power of the 2-DE-based technology was recognized by the research community early on, and scientists from various disciplines were attracted to the field of proteomics. The information obtained by the 2-DE-based approach is high because a number of specific protein attributes can be determined [19]. Thousands of proteins can be resolved and visualized simultaneously on a single 2-DE gel; for each protein, the isoelectric point, MW, and the relative quantity can be measured [20]. High-resolution capabilities of 2-DE allow the separation and detection of post-translationally modified proteins. In many instances, post-translationally modified proteins can be readily located in 2-DE gels because they appear as distinctive horizontal or vertical clusters of spots [21]. In addition, modified proteins can be revealed by MS analysis, when multiple spots of the same protein are identified. In terms of equipment, the 2-DE-based technology is well suited for research conducted in an academic setting [22-23].

MALDI-TOF-MS remains an important tool for protein identification because of its high throughput, sensitivity, and high mass accuracy [24]. Numerous advancements have been made in MALDI-TOF instrumentation and new-generat ion, automated MALDI-TOF mass spectrometers are commercially available [25]. These high throughput systems are run without operator intervention, and incorporate algorithms for iterative optimization of instrument parameters during data acquisition. Improved software tools for the detection of monoisotopic peaks in MALDI-TOF spectra have also been developed [26]. Another type of newly developed MS instrumentation combines electrospray ionization (ESI) with a quadrupole time-of-flight (QTOF) analyzer [27]. The QTOF analyzer can be coupled with MALDI, and MALDI-QtOF-MS was shown to be a promising new tool for proteomics [28]. The latest generation of proteomics instrumentation also includes the MALDI tandem-time-of-flight (MALDI-TOF/ TOF) mass spectrometer. The major advantages of the MALDITOF/ TOF instrument are ultra-high throughput, high sensitivity, and high-energy collision-induced dissociation capabilities that provide enhanced peptide-sequence information [29]. 2-dimensional gel electrophoresis has been used in cancer proteomics, but this technique enabled analysis of only the most abundant proteins and generally with low quantitative accuracy. Mass spectrometry–based proteomics, particularly in a high resolution and quantitative format, has developed rapidly over the last few years [30]. Hybrid mass spectrometers-such as the linear ion trap-Orbitrap-combine high resolution, high mass accuracy, and high peptide sequencing speed [31]. Together with innovations in sample preparation and computational proteomics, these technologies can enable confident peptideand protein identification and quantification at a large scale. Examination of the signature proteins in gene expression studies of large patient cohorts identified IDH2 and CRABP2 as markers of poor prognosis and SEC14L2 as a marker of good prognosis [32].

In recent years, innovations in high-throughput proteomic profiling approaches have allowed for highly sensitive, accurate, and quantitative identification of altered proteins in multiple samples at the same time. Isobaric tags for relative and absolute quantitation (iTRAQ) has been used successfully for the characterization of protein bio indicators of diverse effects [33]. In general, modern proteomic studies often use gel electrophoresis and chromatography combined with MS. Mostly, gel electrophoresis and chromatography are used for separation of protein mixture into [34]. 2-DE investigations showed elevated levels of acute phase proteins such as haptoglobin (_-chain), serum amyloid P, _1-antitrypsin, _1-antichymotrypsin and _1-acidic glycoprotein in plasma from patients diagnosed with breast cancer [35]. Other recently identified breast cancer biomarkers using SELDI include Hsp27, 14-3-3 sigma, and mammaglobin/ lipophilin B complex [36].

Table 1: *The results of modern proteomic studies of BC.*

	Biological samples	Research methods	Methods of validation	Protein(s)
Invasive object	Tumor tissue of invasive ductal carcinoma Subtypes: Luminal B HER2+ve	2D-PAGE (SDS-PAGE), 2D-DIGE, iTRAQ-MD-LC-MS/MS (MD-LC (SCX-LC), MALDI-TOF/TOF MS)	Western blotting MRM-MS	Apolipoprotein A1 (APOA1) Gelsolin (GELS); Heat shock protein HSP 90-beta (hs90b); Eukaryotic elongation factor 1 alpha (EF1A1); Peroxiredoxin 3 (PRDX3); NHRF1.
	HER2 enriched			3 (PRDX3); NHRF1. Peroxiredoxin 1 (PRDX1); Oxidoreductase (catD); Calreticulin (CALR) ATPase beta chain (atpB); SOX14 (CH60) SRY-box 14.
	Tumor tissue of invasive ductal carcinoma Stages: Early stages	2D-PAGE (SDS-PAGE), 2D-DIGE, iTRAQ-MD-LC-MS/MS (MD-LC (SCX-LC), MALDI-TOF/TOF MS)	Western blotting MRM-MS	Tropomyosin 4 (TPM4); Oxidoreductase (catD); Peroxiredoxin 3 (PRDX3); Annexin A3 (ANXA3); Heat shock protein family B (small) member 1 (HSPB1).
	Late stages			Calreticulin (CALR); Ovotransferrinlike (TRFE); Gelsolin (GELS); SOX14 (CH60) SRY-box 14; Capping actin protein, gelsolin like (CAPG); Ywhag (1433G) tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein gamma; Glucose regulated protein 78 (grp78); NHRF1.
	Lymph node positive vs. negative, low grade primary BC tissues	2D-PAGE (SDS-PAGE)	qPCR (transcript level), iTRAQ-2D-LC-MS/MS, mTRAQ-SRM MS, IHC/TMA;	Transgelin (TAGLN)
	Primary breast carcinoma tissues from patients with different lymph node status	iTRAQ-2D-LC-MS/MS	mTRAQ-SRM MS, IHC/TMA	Transgelin (TAGLN); Transgelin-2 (TAGLN2)
	Breast ductal carcinoma tissues	Published data and database (mRNA level)	IHC/TMA	Kinesin associated protein 3 (KIFAP3)
	Metastatic BC (tumor tissue)	Published data and database (mRNA level)	IHC/TMA	Ribosome binding protein 1 (RRBP1)
	Breast tumor tissues HER2+ TNBC	LC-MS/MS (SELDI MS)	IHC	KRT19 (CK19) keratin 19. RNA-binding Ras-GAP SH3 binding protein (G3BP)
	Human disease-free breast tissues and malignant breast tumors	LC-MS/MS with isotope dilution	SRM-MS	Apurinic/apyrimidinic endonuclease 1(APE1)
	BC tissues with different ER, PR and HER2 status (metaanalysis)	Published data on proteins as important targets and proteomic processes in BC	RPPA	ER; PR; Apoptosis regulator (BCL2); GATA binding protein 3 (GATA3); KIAA1324 (EIG121); Epidermal growth factor receptor (EGFR); Erb–b2 receptor tyrosine kinase 2 (HER2); HER2p1248; Cyclin B1 (CCNB1); Cyclin E1 (CCNE1).

Non-invasive object	Serum (patients with recurrent BC and patients with no sign of recurrence 5 years after diagnosis)	Lectin affinity chromatography, 2D-DIGE, LC-MS/MS (RP-LC)	ELISA	CDH5 (CADHERIN5) cadherin 5, type 2 (vascular endothelium)
	Plasma (healthy donors and BC patients)	LC-MS/MS (RP-LC, ESI MS, XCT II MS (TripleQ MS)	MRM-MS	Apolipoprotein A1 (APOA1); Hemopexin hemopexin-like; Angiotensin preprotein.
Combined object	Urine and tumor tissue (identification) Cell lines (validation) Tumor tissue (validation	LC-MS/MS (RP-LC)	Western blotting IHC, Western blotting	Extracellular matrix protein 1 (ECM1); FLG2 (FILAGGRIN) filaggrin family member 2; Microtubule associated serine/threonine kinase family member 4 (MAST4); Microtubule associated serine/ threonine kinase family member 4 (MAST4).

Diagnostic marker protein profiling studies

The goal of mass spectrometry-based protein profiling studies performed for breast cancer is to identify novel diag¬nostic markers [37]. For genetic breast cancer classifications, the sporadic breast cancer subgroups constitute approximately 90% of cases and hereditary cases constitute approximately 10% [38]. With the improvement of MS technologies and sample preparation protocols, the size of cohorts and the quality of proteomic data significantly improved [39]. Liu et al [40] analyzed a cohort of 126 TNBC breast cancer samples using laser capture microdissection liquid chromatographers/MS approach. The total protein coverage obtained was >3500 proteins, and they identified an 11-protein signature for TNBC with 10 proteins that were up-regulated (CMPK1, AIFM1, FTH1, EML4, GANAB, CTNNA1, AP1G1AP1M1, and CAPZB), and one was down-regulated (methylenetetrahydrofolate dehydrogenase 1) in good prognosis patients. The signature presented high predictive value of patient prognosis with area under the curve of 0.83 of a receiver operating characteristics curve. With the use of the same techniques De Marchi et al [41]. obtained a four-protein signature (programmed cell death protein 4, cingulin, ovarian carcinoma immune reactive antigen domain containing protein 1, and Ras GTPase-activating protein binding protein 2), which predicts tamoxifen-susceptibility in recurrent breast cancer. The cohort consisted of 112 ER positive tumor samples with total coverage of 4000 proteins [42].

Using combined LC-MS/MS and bottom-up strategy, protein biomarkers were identified in urine of breast cancer patients with different disease stage and tumor material was studied in parallel as well [43]. Expression levels of 59 proteins was found to be different from that in control samples, in particular, 13 novel up-regulated proteins associated with breast cancer of diagnostic value have been revealed. The relation between breast cancer progression and a panel of specific protein markers has been ascertained: pervasive ductal carcinoma in-situ - leucine LRC36, protein MAST4 and uncharacterized protein CI131, early invasive breast cancer - DYH8, HBA, PEPA, MMRN2 proteins, filaggrin, and uncharacterized protein C4orf14 (CD014), and metastatic breast cancer - AGRIN, NEGR1, FIBA proteins and KIC10 keratin These data will be used for development of screening programs [44]. Predictive protein markers of different breast cancer subtypes will allow us to determine therapeutic response to particular treatment, to optimize and personalize cancer therapy [45]. the following proteins were found to be overexpressed: transketolase, transferrin, CK19, thymosin β4, and thymosin β10. The number of proteins, namely, enolase, peroxiredoxin 5, periostin precursor, cathepsin D preproprotein, vimentin, Hsp 70, annexin 1, RhoA were related to the tumor response to neoadjuvant chemotherapy. Also, two proteins for classification of these subtypes were validated (see Table 1) [44].

In addition, in other study reported an integrated cell line-based discovery based on iTRAQ approach for the identification of protein biomarkers [46]. Further filtering for secreted proteins and prioritization based on gene expression data and immunohistochemically staining from breast cancer tissues combined with iTRAQ results provided a short list of 5 proteins. then assessed their expression level in a verification cohort of 56 samples. authors confirmed the significantly higher concentrations of KLK6, FST, LIF and IGFBP2 in the breast cancer group compared to the healthy controls, whereas tPA expression showed no significant difference between both groups. When an independent validation cohort of 241 invasive breast cancer serum samples and 112 healthy control samples was used, only KLK6 and FST protein expressions were found to be significantly higher in the breast cancer group, compared to healthy controls. Based on these findings, it is proposed that KLK6 and FST could be considered as relevant breast cancer biomarkers that could be tested in future systematic and multi-institutional trials to investigate their clinical utility [47]. Another study data identified CRABP2 and IDH2

as markers of poor prognosis and SEC14L2 as a marker of good prognosis and suggest additional markers that require further evaluation [48].

In one study, the differentially expressed proteins between 23 paired primary breast tumor and metastatic lymph nodes were identified by quantitative iTRAQ proteomic analysis. Immunohistochemistry was applied to locate and assess the expression of NUCB2 in paired primary breast tumor and metastatic lymph node tissues. The results show that NUCB2 (Nucleobindin-2) expression was down-regulated in metastatic lymph node tissues compared with primary breast tumors [49]. Dowling et al. Combined metabolomics and proteomics platforms to analyze cancer and non-cancer serum samples. high mobility group protein HMG-I/HMG-Y (HMGA1) abundance level was found to be associated with breast cancer clinic pathological features [50]. Naif abdullah al-dhabi, normal and tumor tissues were collected from 20 people from a local hospital. Proteins from the diseased and normal tissues have been investigated by 2D gel electrophoresis and MALDI_TOF_MS fingerprint data were fed into various public domains like mascot, MS-fit, and pept-ident against SWISS_PROT protein database and the proteins of interest were identified. Some of the differentially expressed proteins identified were human annexin, glutathione s-transferase, vimentin, enolase-1, dihydrolipoamide dehydrogenase, glutamate dehydrogenase, cyclin A1, hormone sensitive lipase, beta catenin [51].

Based on Reverse phase protein arrays, functional protein classification, subtype differences were noted in Invasive lobular cancer. 6 proteins were statistically different between the RPPA- defined luminal A subgroups: cleaved caspase 9, 53BP1, ampka, GATA3, rad51 and p90rsk thre359/ser363 [52]. Moreover, such a comparison can be explored to find potentially new protein biomarkers for early disease detection. In one study, exosomal proteomes of MDA-MB-231, a metastatic breast cancer cell line, and MCF-10A, a non-cancerous epithelial breast cell line, were identified by nano-liquid chromatography coupled to tandem mass spectrometry. Three exosomal membrane/surface proteins, glucose transporter 1 (GLUT-1), glypican 1 (GPC-1), and disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), were identified as potential breast cancer biomarkers [53]. In 2020 kosok and et al , report identification of specific proteome expression profiles pertaining to two TNBC subclasses, basal A and basal B, through in-depth proteomics analysis of breast cancer cells .they identified kinases AXL, PEAK1, and TGFBR2 and proteases FAP, UCHL1, and MMP2/14 as specific targets for basal B subclass, which represents the more aggressive TNBC cell lines [54].

Conclusion

Approximately 10-15% of patients with breast cancer have an aggressive) disease and develop distant metastases within 3 years after the initial detection of the primary tumor. As it is not possible to accurately predict the risk of metastasis development in individual patients, 80% of the patients received adjuvant chemotherapy, among which 40% relapse and ultimately die of metastatic breast cancer [55]. Thus, we need to identify effective biomarkers or establish metastatic models to predict the occurrence of breast cancer metastasis to provide a better treatment for these patients. At present, many researches are focused on the different proteins of the primary tumor between breast cancer patients with or without lymph node metastases [56]. or exploring the different proteins between cell lines with different metastatic potential [57-60]. Analysis of proteins expressed by serum, plasma and tumors, using novel concepts and methods, should accelerate our quest to attain this goal and bring to light a better and more comprehensive view of the molecular heterogeneity of breast cancers [61]. In this way the proteomics approaches provide powerful tools to study pathological processes or clinically important problems at the molecular level and will have a major impact in the future. Since the introduction of proteomics, 2- DE, SERPA approach and MS have been successfully used in a large number of studies in many biological fields [22]. 2-D electrophoresis coupled with MALDI-TOF/TOF is suitable tool in protein identification because of its relative simplicity and overall visualization of the proteins in the selected pH range. The combination of 2D electrophoresis with the HLPC system enables the enlargement in the number of identified proteins, their sequence coverage and unique identification of various protein isoforms [62].

The study of Breast cancer proteome is directed on profiling of various biologic materials and is aimed at the improvement of prophylaxis, screening, diagnostics, prognosis, and therapy [63]. A large pool of proteins of mammary gland tumors and Breast cancer -associated proteins from body fluids have been already identified, and in part they were validated [64]. The progress of validation methods is helpful in more efficient application of Breast cancer biomarkers in clinical practice [44]. Taken together, the results of proteomics studies demonstrate an integrated interaction of the data and “omics” sources with the systemic approach for assessment of functions of biomolecules in various pathologies and Breast cancer in particular [65].

Limitations

It is clear that more research is needed.

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