Proteomic analysis of infiltrating ductal carcinoma tissues by coupled 2-D DIGE/MS/MS analysis

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INTRODUCTION

There is growing interest in protein expression profiling aiming to identify novel diagnostic markers in breast cancer. Proteomic approaches such as 2-D DIGE/MS/MS have been used successfully for the identification of candidate biomarkers for screening, diagnosis, prognosis and monitoring of treatment response in various types of cancer. Identifying previously unknown proteins of potential clinical relevance will ultimately help in reaching effective ways to manage the disease. From all breast cancers classified, invasive carcinomas represent 70–80% and include more than 10 different types. Among these, infiltrating ductal carcinomas (IDC) are the most aggressive forms associated with poor prognosis.

AIM OF THE STUDY

The aim of this study was to identify specific proteins in infiltrating ductal carcinomas, whose expression is distorted.

MATERIALS AND METHODS

We analyzed breast cancer tissues from five tumor and five normal tissue samples from ten breast cancer subjects with IDC by 2-D DIGE using two types of IPG strips: pH 3–10, 24 cm (DIGE1) and pH 4–7, 24 cm (DIGE2). Tissue samples were chosen based on the type of tumor (Carcinoma ductale invasivum), stage (IIA) and TNM classification (pT2, pN1a, pMx). DIGE images were analyzed using Image Master Platinum software 7.1 (GE Healthcare). Statistically significant, differentially expressed proteins were selected based on two criteria: Annova < 0.05 and Ratio > 2. Protein identification was done by MALDI-TOF-TOF 4800 mass spectrometer (ABI). Database searching was carried out using Mascot version 2.2 via GPS explorer software (ABI) combining MS and MS/MS interrogations on Human proteins from Swiss-Prot databank .

RESULTS

From all the spots detected in both DIGE assays, differentially expressed were 50 spots (position indicated in Figure 1A). Of these, 39 proteins were successfully identified by MS, representing 29 different proteins (Table 1). Among identified spots, 9 were found in both DIGE 1 and DIGE 2 (Figure 1B) and one protein was identified in two different spots in DIGE 2 (spot 9 and 10). Ten proteins were overexpressed in the tumor samples (highlighted in red in Table 2). The 2-D DIGE/MS/MS analysis revealed an increased expression in tumor samples of few proteins not previously associated with breast cancer, such as: CAPG, PMM2, MRI1, FKBP4, CRABP2, lamin B1 and KRT8. The insertion of our data set in Ingenuity Pathway Analysis (IPA) yielded a p-value of 10^{-26} (associating the functions ''Cellular Growth and Proliferation, Cancer, Cell Cycle'')

CONCLUSION

The proteins that we have identified in this study appear to be involved in multiple and diverse pathways and have established roles in cellular metabolism. The possible role of these proteins and their connection with the signal transduction cascade of breast cancer remains to be unsolved in the future. The fact that our differentially expressed proteins were clustered with a high rank in the IPA network, indicates that the identified proteins were not random and may interact biologically.

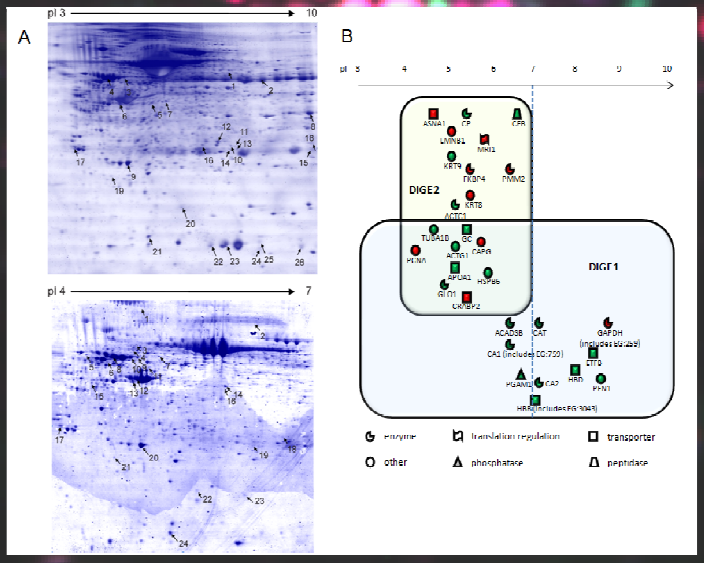

Figure 1. 2-D map of the proteome obtained from breast tissues. (A) Typical preparative Colloidal Coomassie G-250 stained gels with differentially expressed proteins marked with numbered arrows. (B) A map of differentially expressed proteins in IDC, based on type of IPG strip used. Different shapes represent the functional classes of proteins while their color indicates the degree of overexpression (red) or underexpression (green) of the corresponding protein in tumor samples compared to normal samples.

Table 1. List of differentially expressed proteins in IDC identified by MS.

No.		Reference spot	Ratio	Name of the protein in the database SwissProt or NCBInr	Gene	Description	Location	Family
1		Spot01	2.9	Ceruloplasmin	CP	ceruloplasmin (ferroxidase)	Extracellular Space	enzyme
2		Spot02	2.2	Complement factor B	CFB	complement factor B	Extracellular Space	peptidase
3		Spot03	-2.6	Lamin-B1	LMNB1	lamin B1	Nucleus	other
4		Spot04	2.7	Keratin, type I cytoskeletal 9	KRT9	keratin 9	Cytoplasm	other
5		Spot07	-4.5	Peptidyl-prolyl cis-trans isomerase FKBP4	FKBP4	HK506 binding protein 4, 59kDa	Nucleus	enzyme
6		Spot08	4.1	Tubulin alpha-1B chain	TUBA1B	tubulin, alpha 1b	Cytoplasm	other
7		Spot09	2.6	Vitamin D-binding protein	GC	group-specific component (vitamin D binding protein)	Extracellular Space	transporter
8		Spot11	-3.3	Keratin, type II cytoskeletal 8	KRT8	keratin 8	Cytoplasm	other
9	DIGE 1	Spot12	2.4	Actin, alpha cardiac muscle 1	ACTC1	actin, alpha, cardiac muscle 1	Cytoplasm	enzyme
10		Spot13	2.4	Actin, cytoplasmic 2	ACTG1	actin, gamma 1	Cytoplasm	other
11		Spot14	14.9	Macrophage-capping protein	CAPG	capping protein (actin filament), gelsolin-like	Nucleus	other
12		Spot15	-2.8	ATPase ASNA1	ASNA1	arsA arsenite transporter, ATP-binding, homolog 1 (bacterial)	Nucleus	transporter
13		Spot16	-2.9	Methylthioribose-1-phosphate isomerase	MRI1	methylthioribose-1-phosphate isomerase homolog (S. cerevisiae)	Cytoplasm	translation regulator
14		Spot17	-3.5	Proliferating cell nuclear antigen	PCNA	proliferating cell nuclear antigen	Nucleus	other
15		Spot18	-2.1	Phosphomannomutase 2	PMM2	phosphomannomutase 2	Cytoplasm	enzyme
16		Spot20	2.8	Apolipoprotein A-I	APOA1	apolipoprotein A-I	Extracellular Space	transporter
17		Spot21	4.6	Lactoylglutathionc lyase	GLO1	glyoxalase I	Cytoplasm	enzyme
18		Spot23	8.1	Heat shock protein beta-6	HSPB6	heat shock protein, alpha-crystallin-related, B6	Cytoplasm	other
19		Spot24	-6.0	Cellular retinoic acid-binding protein 2	CRABP2	cellular retinoic acid binding protein 2	Cytoplasm	transporter
20		Spot 01	3.0	Catalase	CAT	catalase	Cytoplasm	enzyme
21		Spot 05	2.3	Short/branched chain specific acyl-CoA dehydrogenase, mitochondrial	ACADSB	acyl-CoA dehydrogenase, short/branched chain	Cytoplasm	enzyme
22		Spot 08	2.2	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Cytoplasm	enzyme
23		Spot 10	7.1	Carbonic anhydrase 2	CA2	carbonic anhydrase II	Cytoplasm	enzyme
24		Spot 12	3.0	Carbonic anhydrase 1	CA1	carbonic anhydrase I	Cytoplasm	enzyme
25	DIGE 2	Spot 16	4.7	Phosphoglycerate mutase 1	PGAM1	phosphoglycerate mutase 1 (brain)	Cytoplasm	phosphatase
26		Spot 18	2.4	Electron transfer flavoprotein subunit beta	ETFB	electron-transfer-flavoprotein, beta polypeptide	Cytoplasm	transporter
27		Spot 23	10.4	Hemoglobin subunit beta	HBB	hemoglobin, beta	Cytoplasm	transporter
28		Spot 24	8.7	Hemoglobin subunit delta	HBD	hemoglobin, delta	Cytoplasm	transporter
29		Spot 25	5.5	Profilin-1	PFN1	profilin 1	Cytoplasm	other

Legend: -overexpressed proteins in IDC; -underexpressed proteins in IDC

-reported association with breast cancer

ACKNOWLEDGMENTS

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