Proteome profiling of heat shock of human primary breast epithelial cells, a dataset report

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Abstract Exposure to elevated temperatures has a strong effect on cell functions, and is used in clinical practice. Hyperthermia may affect multiple regulatory mechanisms in cells. To understand better the response to hyperthermia of immortalized primary human breast epithelial cells, we performed a proteomics study of these cells cultured at 34°C or 39°C. Twenty-four proteins were shown to be differentially expressed due to hyperthermia. Analysis of these proteins showed the potential involvement of various biological processes in response to hyperthermia, e.g., cell adhesion, cell communication, and cell cycle. Transforming growth factor- β 2 (TGF- β 2) and heat shock protein 27 (HSP27) were found to be upregulated at 39°C. TGF- β 2 was found to affect expression of HSP27, and to have a protective role in hyperthermia-induced cell death. Thus, the dataset described here of hyperthermia-related proteins in human primary breast epithelial cells predicts a number of cellular activities affected by exposure to high temperatures and provides a set of proteins for further studies.

Keywords Heat shock response · Proteomics · Systemic analysis · Breast primary epithelial cell · Transforming growth factor- β · Heat shock protein 27

Introduction

The heat shock response is a reaction in cells and organisms to elevated temperatures (heat shock or heat stress) (Linguist and Craig 1988). Cells subjected to heat have to be able to adjust to such a shock if they are to survive. The classical responses to heat shock involve the induction of heat shock proteins, which include chaperones and proteases, required for protein refolding and degradation. Stressed cells also require elevated protein synthesis, which facilitates degradation of unwanted proteins. The adjustment of cellular metabolism for optimal growth and survival is another response (Tomanek 2002). The heat shock response has been extensively studied, involving organisms from bacteria, yeast, plant, and animal (Mathew and Morimoto 1998; Burke and Orzech 1988; Stewart et al. 2002). Most studies have focused on the known heat shock proteins (HSPs) family, as well as on the heat shock transcription factor (HSF) family (Pirkkala et al. 2001). Large-scale proteomics studies of heat shock have only been applied to bacterial and marine snails (Movahedi and Waites 2000; Lüders et al. 2009; Tomanek 2005). Relatively, little is known about systemic features of the response to heat shock in human somatic cells. Here, we studied the heat shock response of immortalized primary human breast epithelial cells using a proteomics approach, and identified 24 proteins and explored regulatory signaling pathways that may be involved in the response to heat stress.

Materials and methods

Cells

Human primary epithelial cells were provided by O'Hare et al. (2001). Luminal 226Lts4 and 226LU19 cells were used. The

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cells were immortalized by transfection with catalytic subunit of human telomerase (hTERT). Cultured cells were grown at 34°C, and hyperthermia treatment was carried out by culturing the cells at 39°C ($\pm 0.2^\circ\text{C}$) for 24 h. Culturing of cells at 34°C was performed as this temperature was used for establishing the primary culture (O'Hare et al. 2001). No differences have been observed between these cells cultured at 34°C or 37°C (O'Hare et al. 2001). Therefore, we maintained the culturing temperature 34°C at which the cells were generated, maintained, and characterized as normal human breast epithelial cells. MCF-7 and MDA-MB-231 cells were obtained from ATCC (Manassas, VA, USA), and were cultured in media as recommended by ATCC. Cells were treated with TGF- β 2 (10 ng/ml) (PeproTech, NJ, USA) and with neutralizing pan-specific TGF- β antibody (15 $\mu\text{g/ml}$) (AB-100-NA, R & D Systems, Minneapolis, MN, USA), respectively at 37°C or 39°C for 24 h.

Two-dimensional gel electrophoresis

Samples were dissolved in 2-D GE buffer (8 M urea, 4% CHAPS, 0.5% DTT, IPG buffer, pH 3–10). One hundred micrograms of protein were subjected to isoelectrofocusing (IEF) using 18-cm linear IPG dry strips with a pH range of 3–10 (GE Healthcare, Uppsala, Sweden). IEF was performed in an IPGphor (GE Healthcare, Uppsala, Sweden) using the following protocol: rehydration, 10 h; 50 V, 3 h; 1,000 V, 1 h; 8,000 V, 10 h. After IEF, strips were equilibrated in 50 mM Tris-HCl, pH 8.8, 6 M urea, 2.0% SDS, 30% glycerol with 1% DTT for 10 min, and then for 10 min in the same buffer containing 4% iodoacetamide instead of DTT. Equilibrated strips were placed on top of 10% polyacrylamide gels and were fixed with 0.5% agarose in 62.5 mM Tris-HCl, pH 6.8, 0.1% SDS. SDS-PAGE was performed in a DALTsix following the manufacturer's recommendations (constant power 50 W, for 6–8 h; GE Healthcare, Uppsala, Sweden). Gels were fixed in 10% acetic acid and 20% methanol for 10–12 h. Proteins were detected by silver staining. We generated four 2D gels for each condition.

Image analysis

Silver-stained gels were scanned in an ImageScanner with the MagicScan32 software, and analyzed with calculation of volumes of spots by the ImageMaster 2-D Platinum software (GE Healthcare, Uppsala, Sweden). Protein spots differentially expressed in cells cultured at 34°C and 39°C were considered for protein identification. The statistical significance of changes was evaluated using the ImageMaster 2-D Platinum software; embedded in the software Student's *t* test was used.

Protein identification

Protein spots were excised from gels, destained and subjected to in-gel digestion with trypsin (modified sequence-grade porcine; Promega, Madison, WI, USA). Tryptic peptides were concentrated and desalted on a microC18 ZipTip (Millipore Billerica, MA, USA). Peptides were eluted with 60% acetonitrile containing α -cyano-4-hydroxycinnamic acid as the matrix, applied directly onto the metal target and analyzed on Bruker Ultraflex MALDI TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). Peptide spectra were internally calibrated using trypsin autolytic peptides. To identify proteins, we performed a search of the NCBI database by using the ProFound search engine (<http://65.219.84.5/service/prowl/profound.html>). One miscut and partial oxidation of methionine were allowed. The probability of identification was evaluated according to the probability value (*Z* value) and sequence coverage. Comparison of the experimental *pI* and *Mr* values of proteins to their theoretical values was also considered.

Analysis of data

Systemic analysis of obtained data was performed using GoMiner (<http://discover.nci.nih.gov/gominer/>), Ingenuity Pathway Analysis (IPA; www.ingenuity.com), and Cytoscape tools. GoMiner allows for the classification of identified proteins into biologically coherent categories and assesses these categories. Relations between identified proteins were explored by IPA and Cytoscape. In this report, networks are presented in Cytoscape. For the analysis with Cytoscape, we generated a network using proteins identified by us (Table 1), MiMi tool and KEGG database. The network was viewed in Cytoscape, betweenness was computed by Centiscape tool, and network modules were extracted by MCODE tool. Betweenness is a measure of centrality of nodes; nodes that are on many shortest paths between other nodes have higher betweenness. Degree of centrality is a measure of the number of connections the node has. IPA was used with settings for network analysis as recommended by IPA, e.g., the number of connections and components between two dataset-defined components. Fischer's exact test was used to calculate the *p* value determining the network connectivity.

Immunoblotting

Cell lysates were resolved on SDS polyacrylamide gels and transferred onto Hybond P membranes (GE Healthcare, Piscataway, NJ, USA). Membranes were blocked with 5% (*v/v*) BSA and then incubated with a primary antibody as recommended by the manufacturer, and followed by an

Table 1 List of proteins which change their expression due to high temperature

Spot number	Protein name ^(a)	NCBI accession no. ^a	Probability ^a	”Z”-Value ^a	Sequence coverage (%) ^a	Theoretical value ^a		Experimental value ^b		Expression change
						pI	Mr (kDa)	pI	Mr (kDa)	
1	Cullin homolog 5 (CUL-5) (Vasopressin-activated calcium mobilizing receptor) (VACM-1)	Q93034	1.0e+000	2.43	10	8.4	91.51	7.2	90	Downregulated
2	transforming growth factor beta2	AAA50405	1.0e+000	0.90	15	9.3	48.47	5.3	42	Upregulated
3	FtsJ homolog 1 isoform b	NP_803183	1.0e+000	2.43	13	5.6	36.48	5.2	30	Downregulated
4	Integrin alpha-6 precursor(VA-6) (CD49f)	P23229	1.0e+000	1.10	8	6.4	127.80	7.1	126	Downregulated
5	Chromosome 3 open reading frame 21	AAH19036	1.0e+000	2.43	44	7.9	42.89	8.5	33	Upregulated
6	FKSG11	AAL09963	1.0e+000	2.43	14	6.6	57.26	7.5	65	Downregulated
7	Peroxiredoxin 2 isoform a; thioredoxin-dependent peroxide reductase 1; thiol-specific antioxidant 1; natural killer-enhancing factor B; thioredoxin peroxidase 1; torin	NP_005800.3	1.0e+000	2.42	38	5.7	21.87	5.5	30	Upregulated
8	Solute carrier family 35, member F3	NP_775779	1.0e+000	1.15	13	10.0	55.44	8.2	43	Upregulated
9	Hypothetical protein BC001096	NP_612398.1	9.3e-001	0.59	8	4.6	61.15	7.4	70	Upregulated
10	Pantothenate kinase 1 isoform beta	NP_683879	1.0e+000	1.00	21	6.0	42.28	6.6	30	Downregulated
11	Acetylcholinesterase PI-linked form precursor	NP_056646.1	1.0e+000	1.15	8	6.1	67.81	9.0	80	Downregulated
12	Heat shock 27kD protein	NP_001531	1.0e+000	2.22	43	6.0	22.82	5.8	33	Upregulated
13	Procollagen C-endopeptidase enhancer; procollagen, type 1, COOH-terminal proteinase enhancer	NP_002584.1	1.0e+000	0.63	16	48.78	7.7	7.7	30	Upregulated
14	Unnamed protein product	BAB15602.1	1.0e+000	1.61	18	6.2	82.8	5.5	85	Upregulated
15	Netrin-G1 ligand; brain tumor associated protein LRRC4; NAG14 protein; leucine-rich repeat-containing 4	NP_071426.1	9.5e-001	0.65	19	6.6	73.56	6.9	85	Upregulated
16	Steroid 21-monooxygenase	CAA41707.1	1.0e+000	1.22	15	8.6	42.58	9.1	33	Upregulated
17	CTCL tumor antigen HD-CL-01	AAM44458.1	1.0e+000	1.48	17	8.7	72.34	7.1	73	Downregulated
18	Unnamed protein product	BAB15219	1.0e+000	1.93	28	5.3	87.09	8.1	38	Upregulated
19	Alkylglycerone phosphate synthase precursor	NP_003650.1	1.0e+000	1.57	12	7	73.69	8.8	70	Upregulated
20	Glucagon-like peptide- 1 receptor	AAA17021	1.0e+000	2.43	16	9.1	53.98	7.3	47	Upregulated
21	Unnamed protein product	BAB15602.1	1.0e+000	1.05	14	6.2	82.8	5.9	130	Upregulated
22	Glial fibrillary acidic protein	AAL16662	1.0e+000	2.43	26	5.5	49.85	7.0	30	Upregulated
23	KIAA1364 protein	BAA92602	9.9e-001	0.87	11	6.3	91.71	6.8	105	Downregulated
24	Tyrosine phosphatase epsilon PD 1	CAD23182	8.5e-001	0.43	12	9.6	61.75	5.5	29	Downregulated

^a Protein name, NCBI accession number, probability, ”Z”-value, sequence coverage, theoretical pI and Mr were obtained by search with profound^b Experimental values were calculated from migration position in 2-D gels

Fig. 1 Images of representative 2D gels. Images of 2D gels of proteins of primary human breast epithelial cells cultured at 34°C (**a**) or at 39°C (**b**) are shown. Migration positions of proteins differentially expressed in cells at 34°C and 39°C are indicated by *lines*. The annotation is as in Table 1. pH gradient in the first dimension (isoelectrofocusing) and migration of molecular mass markers in the second dimension are shown at the bottom and on the left side of images, respectively. Images show representative 2D gels out of 4 2D gels generated for each experimental condition and of two separate experiments

HRP-conjugated secondary antibody (GE Healthcare, Uppsala, Sweden). The proteins were visualized using western blotting luminol reagents (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The following antibodies were used, HSP27 (sc-59562, Santa Cruz Biotechnology, Santa Cruz, CA, USA); pan-specific TGF- β (AB-100-NA, R & D Systems, Minneapolis, MN, USA), and actin (sc-1615, C-11, Santa Cruz Biotechnology, Santa Cruz, USA).

Cell proliferation assays

Cell proliferation was measured using a [3 H]thymidine incorporation assay. MCF-7 and MDA-MB-231 cells were

seeded in 48-well plates for proliferation assays. Cells were incubated with 1 μ Ci/ml of [3 H]thymidine for the last 2 h of the 24 h incubation time period. Radioactivity incorporated in DNA was measured as described earlier (Stasyk et al. 2005). The statistical significance of observed differences was evaluated using Student's *t* test.

Cell apoptosis assays

Cell apoptosis assays were determined by using Cell Death Detection ELISA^{PLUS} (Roche, Mannheim, Germany). Briefly, cell lysates were placed in a streptavidin-coated microplate. A mixture of anti-histone-biotin and anti-DNA-POD was added and incubated for 2 h at 25°C. After removal of unbound antibodies by a washing step, POD was determined photometrically at 405 nm with ABTS as a substrate. The statistical significance of observed differences was evaluated using Student's *t* test.

Results

Proteome profiling

The protein expression profiles of primary immortalized human breast epithelial cells cultured under 34°C and 39°C were examined by 2-DE. The experiments were repeated three times with reproducible results; variability between gels of the same experimental condition was less than 10 %, as a number of detected protein spots.

Fig. 2 Cellular functions affected by the identified proteins. The pie chart indicates functions affected by the identified proteins and numbers of assigned proteins. For annotations of proteins, see the text. The pie chart was built on the basis of analysis of the identified proteins using a GoMiner tool. “Cellular functions” category was selected for the analysis of affected functions

Images of representative 2-D gels are shown in Fig. 1. On average, 900 protein spots were reproducibly observed in each gel. Silver-stained protein spots were distributed in all areas of the pH gradient (pH 3–10), and an approximately even distribution was found for proteins in the range of molecular masses from 25 to 100 kDa. The overall distribution of proteins in 2-D gels was similar to proteome patterns observed for other

human breast epithelial cells (Jia et al. 2010; O'Hare et al. 2001).

Forty-five protein spots were observed as differentially expressed between cells cultured at 34°C and 39°C. Twenty-four proteins were unambiguously identified by MALDI-TOF MS, and are discussed further (Fig. 1. and Table 1). Fifteen proteins were found to be upregulated and nine proteins were downregulated in response to elevated temperatures.

Fig. 3 The network formed by the identified proteins. The network exhibits connections of the identified proteins and components directly dependent on them. This interaction network was generated by a MiMi tool and was represented in Cytoscape. MiMi tool was used to retrieve connections between and dependent on the identified

proteins (query interactions between the identified proteins and immediate neighbors). Cytoscape tool was used to represent the network, and plug-ins were used to analyze the network. Nodes are annotated in GO terms, *lanes* represent physical or functional interactions

Systemic analysis of identified proteins

To explore biological processes and functions that could be affected by the identified proteins, we performed a systemic study using GoMiner, Ingenuity Pathway Analysis, and Cytoscape. The GoMiner tool clustered the identified proteins based on various cellular processes. Involvement of the identified proteins in regulation of cell adhesion, cell communication, cell cycle, and cell development was predicted (Fig. 2). An analysis of affected intracellular regulatory mechanisms showed that most of the identified proteins were involved in cellular metabolism (TGFB2, ZFP91, HSPB1, PANK1, GLP1R, AGPS, ACHE, FTSJ1, PTPRE, CUL5, PRDX2). The other functional domains included cell development (ACHE, TGFB2, PRDX2, CUL5, PCOLCE, HSPB1), cell communication (PTPRE, ANPP5A, ACHE, ITGA6, GLP1R, TGF β 2), cellular component organization (ITGA6, TGFB2, ACHE), cell cycle (CUL5, TGFB2), cell proliferation (TGFB2, ACHE, CUL5), cell adhesion (ACHE, ITGA6, TGFB2), and in cell motility (HSPB1, TGFB2, ACHE).

To explore systemic features of the signaling mechanisms represented by the identified proteins, we generated networks of interactions between the identified proteins and proteins and genes that may be affected by the identified proteins. The network generated by MiMi tool showed 170 nodes, including 24 proteins identified by us and 146 proteins they interacted with (Fig. 3). The nodes with highest betweenness were TAF1, HSPB1 (HSP27), HNF4A, ITGA6, HNF4A, GFAP, VIM, MAX, and TGFB (Supplemental Fig. 1a). Analysis of connectivity of nodes showed that TAF1, HSPB1, HNF4A, ITGA6, TP53, CUL5, VIM, GFAP, ITGB, and PXN highly connected nodes, as was revealed by calculation of the node degree (supplemental Fig. 1b). A similar degree of connectivity was observed for a network built using IPA (data not shown). Nine network modules were extracted from the whole network using an MCODE tool embedded in Cytoscape (supplemental Fig. 2). The network modules showed a higher density of interconnections, as compared to the overall degree of connectivity in the whole network. These modules represented protein degradation regulation, TGF β , heat shock response, cell migration, cell proliferation, and cell apoptosis. We found HSPB1 (HSP27), TGF β , SMAD2, CD9, FYN, UBC, MAX, and ACHE were the components with the highest number of connections among the main network modules.

Validation of HSP27 and TGF- β expression in breast epithelial cells by hyperthermia

We validated the changes of expression of two proteins from the heat shock response proteins identified by proteomics (HSP27 and TGF- β). For validation, we used

breast epithelial cells at different stages of transformation, including primary breast epithelial cells, tumorigenic breast cells MCF-7, and metastatic breast cancer cells MDA-MB-231. Total protein extracts from these cells were cultured at different temperatures and immunoblotted with specific antibodies (Fig. 4). The immunoblotting assays showed that HSP27 and TGF- β were upregulated upon 39°C hyperthermia in all three breast epithelial cells mentioned above, which confirmed the protein expression changes observed in the proteomics study (Fig. 4a).

To investigate whether HSP27 expression would be dependent on TGF- β , we treated MCF-7 and MDA-MB-231 cells with TGF- β 2 ligand or TGF- β neutralizing pan-specific antibody. Our data showed that the TGF- β 2 induced HSP27 expression, and the neutralizing TGF- β antibodies decreased HSP27 expression, as compared to

Fig. 4 Induction of HSP27 expression by TGF- β . **a** Validation of expressions of HSP27 and TGF- β proteins. Total extracts of primary human breast epithelial cell, MCF-7 and MDA-MB-231 cells cultured at 37°C and 39°C were subjected to immunoblotting with primary antibodies specific to HSP27, TGF- β , and actin. Purified human recombinant TGF- β 2 was loaded as TGF- β positive control. **b** Representative western blot showed that TGF- β 2 treatment increased and treatment with TGF- β neutralizing antibodies decreased HSP27 expression in MCF-7 and MDA-MB-231 cells at 37°C and 39°C. For 39°C, HSP27 immunoblots are shown after the same exposure as for 37°C at upper panel, and after shorter exposure at lower panel. Representative immunoblots out of three experiments performed (**a**, **b**) are shown

control nontreated cells, both at 37°C and at 39°C, respectively. A stronger difference was observed with cells incubated at 37°C (Fig. 4b). Thus, our results validated the proteomics data related to expression of HSP27 and TGF- β 2, and showed that TGF- β 2 may be a stimulator of HSP27 expression.

TGF- β has a hyperthermia-protective role in breast epithelial cells

To explore the roles of TGF- β in the heat shock response, we treated cells with TGF- β 2 ligand to enhance the effects of TGF- β , or used neutralizing pan-specific TGF- β antibody to decrease the effects of endogenous TGF- β . In Cell Death Detection ELISA^{PLUS} assay, we observed that TGF- β 2 had no significant effects on cell apoptosis at 37°C in both MCF-7 and MDA-MB-231 cell. MDA-MB-231 cells were more sensitive to 39°C heat shock, as compared to MCF-7 cells (Fig. 5a). MDA-MB-231 cells had a significantly higher number of apoptotic cells in all three groups (control, TGF- β 2, Ab-TGF- β), as compared to MCF-7 cells. In agreement with the notion that TGF- β

protects against the cell death-inducing effect of hyperthermia, we observed that the addition of TGF- β 2 to the cells protected them from cell death by 31% and 42% in TGF- β 2-treated MCF-7 and MDA-MB-231 cells at 39°C, as compared to the control group and the TGF- β neutralizing antibody-treated group (Fig. 5a).

To investigate the role of TGF- β on cell proliferation upon heat shock, we performed a [³H]thymidine incorporation assay. We observed that TGF- β 2 inhibited MCF-7 and MDA-MB-231 cell proliferation at 37°C. Hyperthermia decreased cell proliferation rate in both cell lines to the levels observed for the TGF- β 2-treated cells. There was no significant difference in the rate of proliferation between the 37°C and the 39°C TGF- β 2-treated groups (Fig. 5b).

Thus, systemic analysis predicted a number of functions that may be affected by hyperthermia. Our functional studies indicated that TGF- β 2 protected cells from cell death, and that TGF- β 2 stimulated expression of HSP27 in the studied cells. Results reported here also provide a list of proteins, which can be further explored in the context of heat shock response.

Fig. 5 TGF- β role in cell protection by hyperthermia. TGF- β signaling was modulated by treating cells with TGF- β 2 ligand or with neutralizing pan-specific TGF- β antibody, as indicated. **a** Cell apoptosis assays was measured in MCF-7 and MDA-MB-231 cells by Cell Death Detection ELISA^{PLUS}. Cells were treated as indicated

(control, TGF- β 2, Ab-TGF- β). **b** Proliferation rates of MCF-7 and MDA-MB-231 cells were measured by a [³H]thymidine incorporation assay. *p* value and compared conditions are indicated in each panel, as evaluated by Student's *t* test. Representative experiments out of three experiments performed (**a**, **b**) are shown

Discussion

Heat shock is one of the defense mechanisms common to eukaryotic and prokaryotic cells. It may be divided into two types of stress based on its intensity: mild (e.g., temperature 39–42°C) and severe (e.g., temperature 43–45°C). Severe stress is destructive to the cell, causing cell cycle arrest and leading to apoptosis. Adaptation to mild stress protects cells when they are exposed to higher temperatures. Here, we used immortalized human primary breast epithelial cells as a model and observed how their proteome reacted to a mild heat shock stress (39°C). We identified 24 proteins that changed expression upon hyperthermia. Transforming growth factor- β s (TGF- β s) are multifunctional proteins that control proliferation, differentiation, and apoptosis of cells. We observed TGF- β 2 upregulation when the temperature of cell culturing increased to 39°C. It confirmed previous findings with cardiac cells (Karmazyn et al. 1990; Flanders et al. 1993). Our results showed that TGF- β 2 protects cells from apoptosis. Another identified protein in our experiment, HSP27, is also a well-known heat shock protein. HSP27 can inhibit apoptosis by interacting with regulators of apoptosis, and its overexpression has been shown to protect tumor cells from apoptosis (Chen et al. 2007; Beere 2004; Lanneau et al. 2008; Sreedhar and Csermely 2004). We also observed that TGF- β could induce HSP27 expression, which indicated that TGF- β is an upstream regulator of HSP27 (Fig. 4). The possible molecular mechanism may include heat shock factor (HSF) induction of thrombospondin-1 (Yang et al. 2004). Thrombospondin-1 in its turn may activate TGF- β ligands (Schultz-Cherry et al. 1995; Souchelnitskiy et al. 1995). TGF- β -dependent activation of HSP27 was observed in various types of cells but not in the context of hyperthermia (Kwon et al. 2010; Yu et al. 2008; Di et al. 2007). The exact molecular mechanism of TGF- β 2 upregulation upon hyperthermia and TGF- β -dependent induction of HSP27 would require a separate dedicated study. Thus, our validation study showed involvement of TGF- β and HSP27 in response to hyperthermia by human breast epithelial cells.

Several regulatory pathways were found to be potentially affected by hyperthermia. Protein degradation, heat shock response, cell migration, and proliferation were represented by network modules identified in the whole network formed by the identified proteins. HSPB1 (HSP27), TGF β , SMAD2, CD9, FYN, ubiquitylation-regulated proteins, MAX, and ACHE were found as highly connected hubs (supplemental Fig. 2). It suggested that more cellular activities may be involved in retaining the cellular homeostasis upon 39°C heat stress, e.g., apoptosis or necrosis and angiogenesis. Our findings contribute to the description of proteome changes upon hyperthermia, and

presented here the dataset as a source for further functional studies.

Conclusions

Proteome profiling of heat shock of immortalized human primary epithelial cells identified 24 proteins affected by hyperthermia. We validated HSP27 and TGF- β induction in response to heat shock. We also found that TGF- β 2 may have a protective role in heat shock response of breast epithelial cell. The dataset described here indicated the potential involvement of several novel heat shock response regulatory pathways. These findings may contribute to the further understanding of cellular changes as a result of hyperthermia.

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