HHS Public Access

Author manuscript

Arch Dermatol Res. Author manuscript; available in PMC 2016 July 13.

Published in final edited form as:

Arch Dermatol Res. 2012 April ; 304(3): 229–235. doi:10.1007/s00403-012-1210-z.

Cell proliferation and oxidative stress pathways are modified in fibroblasts from Sturge-Weber syndrome patients

Shilpa D. Kadam,

Departments of Neurology and Developmental Medicine, Hugo Moser Kennedy Krieger Research Institute, Baltimore, USA; Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, USA; Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, USA

Marjan Gucek,

Institute for Basic Biomedical Sciences, Johns Hopkins University School of Medicine, Baltimore, USA

Robert N. Cole,

Institute for Basic Biomedical Sciences, Mass Spectrometry and Proteomics Facility, Johns Hopkins University School of Medicine, Baltimore, USA

Paul A. Watkins, and

Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, USA; Department of Neurogenetics, Hugo Moser Kennedy Krieger Research Institute, Baltimore, USA

Anne M. Comi

Departments of Neurology and Developmental Medicine, Hugo Moser Kennedy Krieger Research Institute, Baltimore, USA; Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, USA

Anne M. Comi: comi@kennedykrieger.org

Abstract

Sturge–Weber syndrome (SWS) is defined by vascular malformations of the face, eye and brain and an underlying somatic mutation has been hypothesized. We employed isobaric tags for relative and absolute quantification (iTRAQ-8plex)-based liquid chromatography interfaced with tandem mass spectrometry (LC–MS/MS) approach to identify differentially expressed proteins between port-wine-derived and normal skin-derived fibroblasts of four individuals with SWS. Proteins were identified that were significantly up- or down-regulated (i.e., ratios>1.2 or<0.8) in two or three pairs of samples (n = 31/972 quantified proteins) and their associated p values reported. Ingenuity pathway analysis (IPA) tool showed that the up-regulated proteins were associated with pathways that enhance cell proliferation; down-regulated proteins were associated with suppression of cell proliferation. The significant toxicologic list pathway in all four observations was oxidative stress

Correspondence to: Anne M. Comi, comi@kennedykrieger.org.

Electronic supplementary material The online version of this article (doi:10.1007/s00403-012-1210-z) contains supplementary material, which is available to authorized users.

Conflict of interest The authors do not have a financial relationship with Hunter's Dream for a Cure Foundation.

mediated by Nrf2. This proteomics study highlights oxidative stress also consistent with a possible mutation in the RASA1 gene or pathway in SWS.

Keywords

Sturge–Weber syndrome; Fibroblasts; iTRAQ; Somatic mutation; Nrf2-mediated oxidative stress; Ras pathway

Introduction

Sturge–Weber syndrome (SWS) is a neurocutaneous disorder associated with (1) a facial capillary malformation [port-wine (PW) stain], (2) a dilated capillary–venous vascular malformation in the eye often associated with glaucoma [26], and (3) an ipsilateral vascular malformation of the brain consisting of a paucity of normal cortical draining veins associated with dilated, primarily venous, leptomeningeal vessels [5]. The natural history of the disorder is variable and has been described elsewhere [5].

SWS is thought to be a disorder of abnormal vascular development occurring in early fetal development; however, the underlying cause is unknown. SWS occurs sporadically and a somatic mutation has been proposed as a likely pathogenic mechanism [3]. Indirect evidence suggesting the presence of a somatic mutation in fibroblasts derived from the normal and abnormal skin of patients with SWS has been reported [6, 11]. However, the putative mutation is unknown.

Mutations in the RASA1 gene have been linked with familial capillary malformations in several families [7, 9], most recently in association with Klippel–Trenauney syndrome [10], which is another port-wine birthmark-associated syndrome sometimes seen in conjunction with SWS. The spectrum of vascular malformations associated with RASA1 mutations has been expanding [23]; however, mutations in this gene have yet to be associated with either SWS or sporadically occurring port-wine birthmarks.

This study uses iTRAQ (isobaric tag for relative and absolute quantification) technology to analyze the proteome of early passage fibroblast cell lines derived from skin punch biopsies from affected (port wine birthmark skin) and non-affected (normal skin) of four patients diagnosed with SWS.

Materials and methods

Sample collection

All participants gave informed consent. Punch biopsies were obtained by dermatologists from port-wine skin and normal skin of four individuals with SWS. Primary fibroblast cultures were initiated from each of the eight skin biopsies. Fibroblast cultures were derived at and obtained from the Maryland Brain Bank along with de-identified clinical information. The Institutional Review Board of Johns Hopkins approved the protocol.

Sample preparation

Early passage cryopreserved vials (P1–2) were plated in 100 mm dishes using minimal essential medium (MEM) containing 20% fetal calf serum (FCS), penicillin and streptomycin and maintained at 27°C in 5% CO_2. The next day all plates were fed fresh with 20% FCS MEM. When the first plate became confluent cells were split into normal media (10% FCS MEM). When these P2–P3 plates became 90–100% confluent, they were washed with phosphate-buffered saline (PBS) and cultured in serum-free media for 24 h. Cells were harvested by gentle trypsinization, washed with PBS, resuspended in 0.25 M sucrose buffer containing 10 mM Tris, pH 7.5, 0.1 mM EDTA, and protease inhibitors (Complete, Roche, Germany). Protein concentration was determined by the method of Lowry et al. [15] and each sample was divided into 150 μg aliquots and stored at −80°C.

iTRAQ analysis

Extracted peptides from harvested samples were resuspended in 20 μL 0.5 M TEAB (triethylammonium bicarbonate buffer). Each sample was labeled for 2 h at room temperature by adding 25 μl of one iTRAQ reagent (113, 115, 117 or 121), maintaining the pH between 7.5 and 8.0 with 0.5 M TEAB. All four iTRAQ-labeled samples were then combined and dried. Peptides were fractionated using SCX and stored at −80°C until LC–MS/MS analysis.

Ingenuity pathway analysis of data sets

Raw data obtained from iTRAQ analysis was uploaded and analyzed for sub-cellular location, comparative proteomics, biomarkers and functional networks using proteome software ingenuity pathway analysis (IPA 7.5, Ingenuity Systems®, Redwood City, CA; http://www.ingenuity.com). The entire data set of ratios of identified proteins in port-wine sample to their controls (N) was uploaded onto IPA 7.5. Each identifier was mapped to its corresponding gene object in the Ingenuity knowledge base. Ratios with >1.2-fold change compared to their own control, and with significant *p* values for the number and size of the peptide sequences matched belonging to the protein identified, were set to identify genes whose expression was significantly differentially regulated for each patient. These genes, called focus genes, were overlaid onto a global molecular network developed from information contained in the Ingenuity knowledge base. *Networks* (see Supplementary Tables 1–4) of these focus genes were then algorithmically generated based on their connectivity. The *functional analysis* (see Supplementary Tables 1–4) identified the *biological or toxicity functions* and/or diseases that were most significant to the data set. *Tox lists* (see Supplementary Tables 1–4) identified functionally grouped gene sets and pathways that describe critical pathways and key adaptive, defensive, or reparative responses resulting from insults from the ingenuity pathways analysis library. The *functional analysis* of a network identified the biological functions and/or diseases that were most significant to the genes in the network. The significance of the association between the data set and the lists were measured in two ways: (1) a ratio of the number of genes from the data set that met the expression value cutoff that map to the list divided by the total number of molecules that exist in the list is displayed. (2) Fischer's exact test was used to calculate a *p* value

determining the probability that the association between the genes in the data set and the list is explained by chance alone.

Results

Table 1 displays the limited demographic and clinical information available for the early passage fibroblast cultures. Three of the subjects were female and three were children; ages ranged from 5 to 32 years. Three of the subjects had seizures, three reported weakness on one side of the body (hemiparesis) and three were taking anticonvulsants. Two were reported to have glaucoma, and endocrine issues were reported in one subject. One subject was known to have had laser treatment to that region; in one subject laser status was unknown.

Table 2A displays the 16 proteins which were increased (>1.2) in the port-wine fibroblast in two of the pairs. Table 2B displays the 13 proteins which were decreased (<0.8) in the port-wine fibroblast in two of the pairs. Table 2C displays two proteins which were increased or decreased in three of the pairs. Consistently increased proteins included several ribosomal proteins and proteins that support cellular proliferation such as caveolin-1, high mobility group protein B1, and prothymosin alpha (ProTα). Consistently decreased proteins included GTP-binding protein SAR1b [12], MAPK 3, rho-GTPase-activating protein 1, S100-A6 [29], and methionyl-tRNA synthetase cytoplasmic [14], that are involved in control of cellular proliferation. Supplemental Table 1 includes all the protein ratios for all four pairs of samples compared.

Oxidative stress mediated by nuclear factor-erythroid 2-related factor 2 (Nrf2) was significant in all four observations for the top toxicological list. Table 3 indicates all the proteins significantly regulated from the Nrf2-mediated oxidative stress response pathway in this data set. Oxidative stress, aryl hydrocarbon receptor (AhR) signaling and hepatic fibrosis were all significant in three of four observations.

Discussion

In the network analyses, the disease process of cancer was significant and in the sub-category of physiological system development and function, tumor morphology was significant. Several consistently increased proteins in the port-wine-derived fibroblasts are known to support cellular proliferation, while a common theme in the consistently decreased proteins is control of cellular proliferation. Rho-GTPase-activating protein 1 was decreased in all four ratios and significantly so in two of the pairs. Rho-GTPase-activating protein 1 is a potent tumor suppressor that interacts with RASA1 [30]. In addition, Rho-GTPase-activating protein 1 silencing in epithelial cells promoted pro-angiogenic responses [24]. RASA1 regulates neurite outgrowth through its interactions with the ephrins [8] and regulates proliferation partly through regulating MAP kinases. The RASA1 protein, however, is not included on the panel of screened proteins. Molecules from the MAP kinase family were consistently down-regulated. MAP kinases, act in a signaling cascade that regulates proliferation, differentiation, and cell cycle progression in response to a variety of extracellular signals. In fibroblasts they mediate the proliferative action of multifunctional

cytokine p43 which acts on endothelial and immune cells to control angiogenesis and inflammation [20].

Oxidative stress response mediated by Nrf2 was significant in all four observations (Table 3). Upon exposure of cells to oxidative stress, Nrf2 is phosphorylated in response to protein kinase C, phosphatidylinositol 3-kinase, and MAP kinase pathways. Nrf2 then binds to the antioxidant response elements within the promoter of enzymes, such as glutathione *S*-transferase, NAD(P)H quinone oxidoreductase, heme oxygenase and superoxide dismutase, and activates their transcription. Aryl hydrocarbon receptor signaling and oxidative stress were also significant in three of the four toxicological lists. AhR is a ligand-activated factor of the basic helix-loop-helix/Per-arnt-Sim family with important roles in metabolic adaption and in organ and vascular development [2, 13, 22]. AhR and Nrf2 have been shown to function coordinately in protection against oxidative stress. Interestingly, all the cultures were responding to the relative stress of being in a serum-free medium for 24 h; it is possible, that this culture condition brought out some of the changes noted in the comparisons.

Prothymosin alpha, another up-regulated gene in this study, has been shown to be one of the hypoxia-induced genes that is up-regulated in areas where the local blood supply is poorly organized, occluded, or unable to keep pace with the growth of cells [4]. Studies indicate that ProTα plays in vivo neuroprotective roles after ischemic events [27]. ProT α is also thought to play a role in cell proliferation, carcinogenesis and apoptosis [28]. Brain abundant membrane attached signal protein 1(BASP1) was significantly modulated in all paired samples tested, is involved in apoptotic cell death pathways [19], and its down-regulation may also indicate a pro-proliferative out-come. Deficient growth hormone (GH) signaling down regulates BASP1. This is of interest since a subset of SWS patients show GH deficits [16]. BASP1's role in extracellular matrix interaction for neurite outgrowth [1, 17, 18] is also of interest for SWS; deficits in the number of perivascular nerves detected in skin biopsy samples of SWS patients have been hypothesized to underlie the pathogenesis of port-wine stains [21, 25].

No single protein was differentially expressed in the same direction in all four pairs of observations; this lack of consistency could result from (a) differences in the percentages of mutated cells or (b) different underlying mutations. Limitations of this study include the limited clinical data available with these fibroblast samples, as well as the small number of subjects. The rarity of SWS increases the difficulty in obtaining both skin biopsies and fibroblasts from these subjects.

Conclusion

Future studies are needed to determine the clinical relevance of these findings; however, this proteomics study creates a roadmap for next steps. The consistent pathways identified direct our attention to a possible dysregulated response to oxidative stress in the SWS/port-wine-derived fibroblasts. This hypothesis can be addressed by quantitatively assaying (1) cellular proliferation and (2) oxidative stress responses in these SWS fibroblast cultures. Furthermore, these proteomics results are consistent with a mutation in the RASA1 gene or

pathway. Therefore, genetic screening for a somatic mutation in the RASA1 gene, in fibroblasts or tissue taken from port-wine birthmarks in SWS, should be pursued.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge grant support from Hunter's Dream for a Cure and assistance obtaining samples from the Sturge–Weber Foundation and the Maryland Brain Bank. We thank Aditya K. Sreenivasan and Kira Lanier for editing this manuscript, and subjects who donated tissue.

References

1. Aigner L, Arber S, Kapfhammer JP, Laux T, Schneider C, Botteri F, Brenner HR, Caroni P. Overexpression of the neural growth-associated protein GAP-43 induces nerve sprouting in the adult nervous system of transgenic mice. Cell. 1995; 83:269–278. [PubMed: 7585944]
2. Bock KW, Kohle C. Ah receptor: dioxin-mediated toxic responses as hints to deregulated physiologic functions. Biochem Pharmacol. 2006; 72:393–404. [PubMed: 16545780]
3. Bodensteiner JB. Sturge–Weber syndrome. Facial Plast Surg Clin North Am. 2001; 9:569–576. [PubMed: 17590943]
4. Burke B, Giannoudis A, Corke KP, Gill D, Wells M, Ziegler-Heitbrock L, Lewis CE. Hypoxia-induced gene expression in human macrophages: implications for ischemic tissues and hypoxia-regulated gene therapy. Am J Pathol. 2003; 163:1233–1243. [PubMed: 14507633]
5. Comi, AM.; Roach, ES. Neurologic manifestations of Sturge–Weber syndrome. In: Bodensteiner, J.; Roach, ES., editors. Sturge–Weber syndrome. Sturge–Weber Foundation; Mount Freedom: 2010. p. 27-38.
6. Comi AM, Hunt P, Vawter MP, Pardo CA, Becker KG, Pevsner J. Increased fibronectin expression in Sturge–Weber syndrome fibroblasts and brain tissue. Pediatr Res. 2003; 53:762–769. [PubMed: 12621118]
7. Eerola I, Boon LM, Mulliken JB, Burrows PE, Dompmartin A, Watanabe S, Vanwijck R, Vikkula M. Capillary malformation-arteriovenous malformation, a new clinical and genetic disorder caused by RASA1 mutations. Am J Hum Genet. 2003; 73:1240–1249. [PubMed: 14639529]
8. Elowe S, Holland SJ, Kulkarni S, Pawson T. Down regulation of the Ras-mitogen-activated protein kinase pathway by the EphB2 receptor tyrosine kinase is required for ephrin-induced neurite retraction. Mol Cell Biol. 2001; 21:7429–7441. [PubMed: 11585923]
9. Hershkovitz D, Bercovich D, Sprecher E, Lapidot M. RASA1 mutations may cause hereditary capillary malformations without arteriovenous malformations. Br J Dermatol. 2008; 158:1035–1040. [PubMed: 18363760]
10. Hershkovitz D, Bergman R, Sprecher E. A novel mutation in RASA1 causes capillary malformation and limb enlargement. Arch Dermatol Res. 2008; 300:385–388. [PubMed: 18327598]
11. Huq AH, Chugani DC, Hukku B, Serajee FJ. Evidence of somatic mosaicism in Sturge–Weber syndrome. Neurology. 2002; 59:780–782. [PubMed: 12221183]
12. Jardim DL, da Cunha AF, Duarte AS, dos Santos CO, Saad ST, Costa FF. Expression of Sara2 human gene in erythroid progenitors. J Biochem Mol Biol. 2005; 38:328–333. [PubMed: 15943909]
13. Kohle C, Bock KW. Coordinate regulation of phase I and II xenobiotic metabolisms by the Ah receptor and Nrf2. Biochem Pharmacol. 2007; 73:1853–1862. [PubMed: 17266942]
14. Liu J, Chung HJ, Vogt M, Jin Y, Malide D, He L, Dundr M, Levens D. JTV1 co-activates FBP to induce USP29 transcription and stabilize p53 in response to oxidative stress. EMBO J. 2011; 30:846–858. [PubMed: 21285945]

15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193:265–275. [PubMed: 14907713]
16. Miller RS, Ball KL, Comi AM, Germain-Lee EL. Growth hormone deficiency in Sturge–Weber syndrome. Arch Dis Child. 2006; 91:340–341. [PubMed: 16551788]
17. Mosevitsky MI. Nerve ending “signal” proteins GAP-43, MARCKS, and BASP1. Int Rev Cytol. 2005; 245:245–325. [PubMed: 16125549]
18. Mosevitsky MI, Novitskaya VA, Plekhanov AY, Skladchikova GY. Neuronal protein GAP-43 is a member of novel group of brain acid-soluble proteins (BASPs). Neurosci Res. 1994; 19:223–228. [PubMed: 8008250]
19. Ohsawa S, Watanabe T, Katada T, Nishina H, Miura M. Novel antibody to human BASP1 labels apoptotic cells post-caspase activation. Biochem Biophys Res Commun. 2008; 371:639–643. [PubMed: 18457665]
20. Park SG, Shin H, Shin YK, Lee Y, Choi EC, Park BJ, Kim S. The novel cytokine p43 stimulates dermal fibroblast proliferation and wound repair. Am J Pathol. 2005; 166:387–398. [PubMed: 15681823]
21. Parsa CF. Sturge–Weber syndrome: a unified pathophysiologic mechanism. Curr Treat Options Neurol. 2008; 10:47–54. [PubMed: 18325299]
22. Poland A, Knutson JC. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Annu Rev Pharmacol Toxicol. 1982; 22:517–554. [PubMed: 6282188]
23. Revencu N, Boon LM, Mulliken JB, Enjolras O, Cordisco MR, Burrows PE, Clapuyt P, Hammer F, Dubois J, Baselga E, Brancati F, Carder R, Quintal JM, Dallapiccola B, Fischer G, Frieden IJ, Garzon M, Harper J, Johnson-Patel J, Labreze C, Martorell L, Paltiel HJ, Pohl A, Prendiville J, Quere I, Siegel DH, Valente EM, Van HA, Van HL, Vaux KK, Vicente A, Weibel L, Chitayat D, Vikkula M. Parkes Weber syndrome, vein of Galen aneurysmal malformation, and other fast-flow vascular anomalies are caused by RASA1 mutations. Hum Mutat. 2008; 29:959–965. [PubMed: 18446851]
24. Shih YP, Liao YC, Lin Y, Lo SH. DLC1 negatively regulates angiogenesis in a paracrine fashion. Cancer Res. 2010; 70:8270–8275. [PubMed: 20861185]
25. Smoller BR, Rosen S. Port-wine stains. A disease of altered neural modulation of blood vessels? Arch Dermatol. 1986; 122:177–179. [PubMed: 3511859]
26. Sujansky E, Conradi S. Sturge–Weber syndrome: age of onset of seizures and glaucoma and the prognosis for affected children. J Child Neurol. 1995; 10:49–58. [PubMed: 7769179]
27. Ueda H. Prothymosin [alpha] and cell death mode switch, a novel target for the prevention of cerebral ischemia-induced damage. Pharmacol Ther. 2009; 123:323–333. [PubMed: 19500618]
28. Vareli K, Frangou-Lazaridis M. Prothymosin [alpha] is localized in mitotic spindle during mitosis. Biol Cell. 2004; 96:421–428. [PubMed: 15325071]
29. Wei BR, Hoover SB, Ross MM, Zhou W, Meani F, Edwards JB, Spehalski EI, Risinger JI, Alvord WG, Quinones OA, Belluco C, Martella L, Campagnutta E, Ravaggi A, Dai RM, Goldsmith PK, Woolard KD, Pecorelli S, Liotta LA, Petricoin EF, Simpson RM. Serum S100A6 concentration predicts peritoneal tumor burden in mice with epithelial ovarian cancer and is associated with advanced stage in patients. PLoS One. 2009; 4:e7670. [PubMed: 19888321]
30. Yang XY, Guan M, Vigil D, Der CJ, Lowy DR, Popescu NC. p120Ras-GAP binds the DLC1 Rho-GAP tumor suppressor protein and inhibits its RhoA GTPase and growth-suppressing activities. Oncogene. 2009; 28:1401–1409. [PubMed: 19151751]

Table 1

Origin of SWS affected and unaffected skin punch biopsy-derived fibroblast cell lines

Patient iTRAQ ID# PW:N	Age (years)	Sex	Race	History/medication	Biopsy location	Symptoms	Port-wine stain appearance
114:113	5	F	Caucasian	Autism, seizure disorder and SWS, GARS test for autism + Karyotype for fragile X syndrome—negative Depakote	PW: scalp N: neck	Seizures, autism	One previous laser treatment
116:115	13	F	Caucasian	Glaucoma both eyes, hemiparesis, seizures, endocrine problems, normal IQ Xalatan eye drops	PW: near hairline N: behind ear	Seizures, hemiparesis, glaucoma	Flat, pink, no previous laser
118:117	8	F	Caucasian	Glaucoma, hemiparesis, seizures, learning problems, attention problems, migraines, affected eye darker blue Neurontin, Dilantin, Periactin, baby aspirin	PW: behind ear N: neck posteriorly	Seizures, hemiparesis, migraines, learning problems	Flat, pink, unilateral, tissue hypertrophy
121:119	32	F	Caucasian	Hemiparesis, learning problems Neurontin	PW: chin/lip N: forehead	Hemiparesis, learning problems	Raised, red, unilateral, no previous laser

Arch Dermatol Res. Author manuscript; available in PMC 2016 July 13.

Table 2

Proteins up-regulated, down-regulated and dysregulated in three pairs in port-wine samples from two pairs

#	Accession	Name	Score	% Cov	Peptides matched	114:113	116:115	118:117	121:119
(A)									
1	Q02878|RL6_HUMAN	60S ribosomal protein L6	12.0	39.6	**9**	**1.32*****	**1.36*****	0.93	1.09
2	P62701|RS4X_HUMAN	40S ribosomal protein S4, X isoform	11.5	44.5	**5**	**1.23*****	**1.23*****	0.97	1.00
3	P62917|RL8_HUMAN	60S ribosomal protein L8	9.1	42.0	**6**	**1.26*****	**1.25****	1.01	1.03
4	P50914|RL14_HUMAN	60S ribosomal protein L14	5.9	53.1	**4**	**1.32****	**1.38***	0.87	1.06
5	P62899|RL31_HUMAN	60S ribosomal protein L31	5.7	45.6	**1**	**1.29****	**1.21****	0.98	1.03
6	P47914|RL29_HUMAN	60S ribosomal protein L29	2.5	30.2	**1**	**1.24***	**1.29***	1.05	0.97
7	P40429|RL13 A_HUMA	60S ribosomal protein L13a	2.2	51.7	**1**	**1.23***	**1.25****	0.98	1.01
8	Q03135|CAV1_HUMAN	Caveolin-1	16.2	50.0	**11**	0.83	**1.38*****	0.96	**1.22****
9	P62805|H4_HUMAN	Histone H4	10.3	62.1	**7**	**1.24****	0.82	**1.52*****	1.02
10	P09429|HMGB 1_HUM	High mobility group protein B1	8.3	28.8	**4**	**1.37*****	**1.20****	0.87	1.05
11	P25786|PSA1_HUMAN	Proteasome subunit alpha type-1	5.7	20.2	**3**	**1.34***	0.90	**1.33***	0.89
12	P17844|DDX5_HUMAN	Probable ATP-dependent RNA helicase DDX5	5.2	24.4	**2**	**1.31*****	0.99	**1.26*****	0.87
13	P02792|FRIL_HUMAN	Ferritin light chain	4.7	18.9	**2**	1.07	**1.20****	1.00	**1.30****
14	P63241 |IF5A1_HUMAN	Eukaryotic translation initiation factor 5A-1	4.1	51.3	**3**	**1.28*****	**1.32*****	0.95	1.14
15	P06454|PTMA_HUMA	Prothymosin alpha (contains: thymosin alpha-1)	4.0	20.7	**2**	**1.88****	0.92	**2.06****	0.93
16	P24534|EF1B_HUMAN	Elongation factor 1-beta	2.0	20.4	**1**	**1.33***	0.97	**1.68***	0.81
(B)									
1	P|2111|C06A3_HUMAN	Collagen alpha-3(VI) chain precursor	25.9	35.2	11	**0.76*****	1.19	**0.80*****	1.05
2	P08123|COlA2_HUMAN	Collagen alpha-2(I) chain precursor	16.7	37.5	11	**0.79****	1.14	**0.75*****	1.27
3	P04264|K2C1_HUMAN	Keratin, type II cytoskeletal 1	7.8	30.6	4	1.00	**0.73***	0.85	**0.75***
4	Q9Y6B6|SAR1B_HUMAN	GTP-binding protein SAR1b	3.6	23.2	1	**0.73***	0.95	**0.63****	1.02
5	P27361|IMK03_HUMAN	Mitogen-activated protein kinase 3	3.36	23.2	1	**0.64***	**0.48****	**1.20**$^{\#}$	0.89
6	Q07960|RHG01_HUMAN	Rho-GTPase-activating protein 1	3.2	20.5	1	**0.79****	**0.77****	0.87	0.98

#	Accession	Name	Score	% Cov	Peptides matched	114:113	116:115	118:117	121:119
7	Q96S66|CLCC1_HUMAN	Chloride channel CLIC-like protein 1 precursor	2.8	10.9	1	**0.65** *	**0.68** *	1.11	1.01
8	P18085|ARF4_HUMAN	ADP-ribosylation factor 4	2.5	24.4	1	**0.75** *	0.83	**0.73** ***	1.07
9	P06703|S10A6_HUMAN	Protein S100-A6	2.2	16.7	1	**0.72** ***	1.13	**0.75** ***	0.80
10	Q15691|MARE1 _HUMAN	Microtubule-associated protein RP/EB family member 1	2.1	23.5	1	0.94	**0.80** *	1.00	**0.79** *
11	P56192|SYMC_HUMAN	Methionyl-tRNA synthetase, cytoplasmic	2.1	7.2	1	**0.80** *	**0.79** *	1.01	**1.22** #
12	P61204|ARF3_HUMAN	ADP-ribosylation factor 3	2.1	33.7	1	**0.68** *	1.05	**0.62** **	0.95
13	P02768|ALBU_HUMAN	Serum albumin precursor	2.0	5.3	2	**0.47** **	**0.69** *	0.92	0.97
(C)									
1	P80723|BASP_HUMAN	Brain abundant, membrane attached signal protein 1	14.8	70.5	**10**	**0.79** ***	**0.56** ***	**1.52** ***	**0.73** ***
2	Q13123|RED_HUMAN	Protein Red	2.0	9.3	**1**	**1.39** *	**1.24** *	0.84	**1.29** *

Score = protein pilot cumulative ProtScore based on 1, 1.3, or 2 points per each unique 90, 95 or 99%, respectively, confident peptide identification

*** $p < 0.0005$;

** $p < 0.005$;

* $p < 0.05$;

$p > 0.05$

Arch Dermatol Res. Author manuscript; available in PMC 2016 July 13.

Table 3

Nrf2 pathway proteins regulated in the four observations

Symbol	Entrez gene name	GenBank	Fold change	*p* value	Net-works	Location	Type	Entrez gene ID for human
Obs 1 (©2000–2009 Ingenuity Systems, Inc. All rights reserved)								
EPHX1	Epoxide hydrolase 1, microsomal (xenobiotic)	NM_000120	−1.207	4.74E–04	6	Cytoplasm	Peptidase	2052
MAPK1	Mitogen-activated protein kinase 1	NM_002745	−1.284	1.85E–02	2	Cytoplasm	Kinase	5594
MAPK3	Mitogen-activated protein kinase 3	NM_002746	−1.574	1.59E–02	2	Cytoplasm	Kinase	5595
SOD2	Superoxide dismutase 2, mitochondrial	NM_000636	−1.326	1.46E–02	4	Cytoplasm	Enzyme	6648
STIP1	Stress-induced-phosphoprotein 1	NM_006819	1.287	3.42E–04	1	Cytoplasm	Other	10963
UBB	Ubiquitin B	NM_018955	1.262	2.51E–12	1	Cytoplasm	Other	7314
Obs 2 (©2000–2009 Ingenuity Systems, Inc. All rights reserved)								
ACTA2	Actin, alpha 2, smooth muscle, aorta	NM_001613	−1.509	1.39E–07	2	Cytoplasm	Other	59
FTH1	Ferritin, heavy polypeptide 1	NM_002032	1.282	8.95E–06	1	Cytoplasm	Enzyme	2495
FTL	Ferritin, light polypeptide	BC067772	1.202	4.94E–03	1	Cytoplasm	Other	2512
MAPK3	Mitogen-activated protein kinase 3	NM_002746	−2.088	3.97E–03	3	Cytoplasm	Kinase	5595
USP14	Ubiquitin specific peptidase 14 (tRNA-guanine transglycosylase)	NM_005151	1.471	1.46E–02	7	Cytoplasm	Peptidase	9097
Obs 3 (©2000–2009 Ingenuity Systems, Inc. All rights reserved)								
CAT	Catalase	NM_001752	−1.447	2.18E–02	2	Cytoplasm	Enzyme	847
DNAJA1	DnaJ (Hsp40) homolog, subfamily A, member 1	NM_001539	1.497	6.92E–03	2	Nucleus	Other	3301
EPHX1	Epoxide hydrolase 1, microsomal (xenobiotic)	NM_000120	1.353	8.38E–05	5	Cytoplasm	Peptidase	2052
NQO1	NAD(P)H dehydrogenase, quinone 1	NM_000903	1.354	2.55E–02	1	Cytoplasm	Enzyme	1728
RRAS2	Related RAS viral (r-ras) oncogene homolog 2	NM_012250	1.279	6.91E–03	1	Plasma membrane	Enzyme	22800
SOD3	Superoxide dismutase 3, extracellular	NM_003102	1.294	9.87E–03	4	Extracellular space	Enzyme	6649
Obs 4 (©2000–2009 Ingenuity Systems, Inc. All rights reserved)								
ACTA2	Actin, alpha 2, smooth muscle, aorta	NM_001613	1.275	1.49E–07	1	Cytoplasm	Other	59
ERP29	Endoplasmic reticulum protein 29	NM_006817	−1.214	4.52E–02	2	Cytoplasm	Transporter	10961
FTL	Ferritin, light polypeptide	BC067772	1.304	3.30E–03	4	Cytoplasm	Other	2512
MAPK1	Mitogen-activated protein kinase 1	NM_002745	−1.233	2.90E–03	1	Cytoplasm	Kinase	5594