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**Original article**

# Expression profile of human neuroblastoma cells after exposure to *Naegleria fowleri*

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***Background:*** *Naegleria fowleri*, a free-living amoeboflagellate, is the causative agent of life threatening primary amoebic meningoencephalitis with up to a 98% mortality rate. The pathogenesis involves neuronal dysfunction and damages the host central nervous system. Understanding the interactions of the target cells and the protozoa may facilitate an integrated approach to address important biological questions.
***Objective:*** We investigated changes in protein expression of the host cells post *Naegleria fowleri* infection.
***Methods:*** The analysis was based on two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) of the proteins extracted from human neuroblastoma SK-N-MC after exposure to *N. fowleri* trophozoites over a period of six hours. We observed differences in protein expression as early as 15 minutes after the exposure, and the differences remained throughout the six hours study.
***Results:*** The identified protein spots corresponded to 53 proteins unique to human and 24 of these proteins were identified as involved in signal transduction, translation, immune response, cytoskeleton, and stress response. We also observed changes in the abundance of these proteins after cocultivating SK-N-MC with *N. fowleri*, especially those involved in angiogenesis, fatty acid metabolism, signal transduction, translation, stress response, signal pathway, and respiration.
***Conclusions:*** The proteomics analysis from this report suggested how and by which means the SK-N-MC responded after *N. fowleri* infection.

***Keywords*:** Host response trophozoites, human neuroblastoma SK-N-MC, *Naegleria fowleri* infection proteomics

Human primary amoebic meningoencephalitis (PAM) is a rapidly lethal disease of the central nervous system caused by a free-living amoeboflagellate *Naegleria fowleri*, an opportunistic parasite. The first case of PAM was reported by Fowler and Carter in 1965. The disease has been reported worldwide with up to a 98% mortality rate. PAM has been found in healthy patients with history of swimming or diving in freshwater containing the parasite [1]. The parasites damage the central nervous system, in both white and grey matter of the brain and subarachnoid space, resulting in death of the patient within approximately a week after onset.

Studies of cytopathogenesis could provide the knowledge of cellular level in coculture models. Several publications have reported that pathogenesis involved in contact and noncontact mechanisms of the amoebae trophozoites. Previous studies have investigated the interaction of *N. fowleri* with host cells such as Vero, rat microglia, rat neuroblastoma, Chinese hamster ovarian, human microglia, and human neuroblastoma cells, respectively [2-5]. Of these, human neuroblastoma seems to represent an authentic model of human brain tissue and it has been used for experiments with PAM [6].

*N. fowleri* directly damages host cells by trogocytosis, using a food-cup structure on their

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surface and releasing cytolytic molecules such as proteases [7]. *N. fowleri* also causes apoptosis of host cells by releasing factors from complex pathways in a noncontact condition [4]. Although several host cell destructive mechanisms of the amoebae have been reported, the mechanism by which host cells respond to *N. fowleri* infection remains unclear.

Several proteins play some important roles in the cytopathogenesis and host response of *N. fowleri* infection. Cysteine protease, acid hydrolase, elastase, fumarase, neuraminidases, phospoholipases, and specifically naegleriapores A and B produced by *N. fowleri* have been reported to involve the infection [8]. However, a series of cytokines and antibodies are produced by the host cells [2]. In this study, we attempted to elucidate systemic responses of the host cells when being exposed to the parasite by using proteomics as an analytical tool.

The protein expression of the host cells could allow us to understand better the cytopathogenesis of *N. fowleri*. Our objective was to investigate changes in protein profiles in the human neuroblastoma (SK-N-MC) cells after exposure to *N. fowleri* trophozoite. The proteomes of the SK-N-MC were analyzed using two techniques: two dimensional polyacrylamide gel electrophoresis (2D-PAGE) and liquid chromatography mass spectrometry (LC-MS). The 2D-PAGE technique separates proteins in the first dimension with isoelectric focusing (IEF); the proteins become separated according to their isoelectric point. In the second dimension, the proteins separate according to their molecular mass by denaturing gel electrophoresis [9]. LC-MS allows the direct identification of individual constituents of protein complexes. The information yielded from these approaches showed global alteration of protein profiles of the infected cells and could help us to understand better the cytopathogenesis of *N. fowleri*.

## Materials and methods

### *Human neuroblastoma SK-N-MC monolayer cells culture*

Human neuroblastoma cells (SK-N-MC) were purchased from the Cell Line Service (Eppelheim, Germany). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM): HAM's F-12 medium containing 5% heat-inactivated fetal bovine serum (FBS, Gibco, Grand Island, NY,USA) without antibiotics at 37°C in the presence of 5% $CO_2$. Cultivation was performed as described previously [5].

### *N. fowleri culture*

The amoeba *N. fowleri* Siriraj strain, isolated from serospinal fluid of PAM patient at Siriraj hospital, Bangkok, Thailand in 1986, was cultured axenically without agitation in Nelson's medium supplemented with 5% FBS in T75-$cm^2$ polystyrene tissue culture flasks (Corning, Corning, NY, USA) at 37°C until used [5].

### *Co-culture SK-N-MC with N. fowleri trophozoites*

The amoebae were added to T-75 $cm^2$ flasks containing SK-N-MC at a ratio of 1:1. The plates were incubated at 37°C in the presence of 5% $CO_2$ for 6 hours. At 0, 0.25, 1, 2, 3, 4, 5, and 6 hours after the incubation, the plate was incubated at 4°C for 10 minutes. The supernatant was discarded. After washing twice with PBS, the cells were removed from the flask using cell scraper. The cells were centrifuged at 200 *g* at 4°C for 5 minutes and the pellets were stored at –80°C until used.

### *Preparation for IEF*

Total protein was extracted from SK-N-MC using the buffer containing 9 M urea, 2 M thiourea, 4% CHAPS, 2% IPG Buffer, and 18 mM dithiothreitol (DTT), and centrifuged for 15 minutes to pellet the insoluble substances. After determining the protein concentration using a Lowry assay (10), approximately 600 µg of protein were loaded onto an IPG strip and focused using an Ettan IPGphor 3 System (GE Healthcare) with a strip holder as follows, step and hold at 500 V for 500 Vh; gradient to 1000 V for 6000 Vh; gradient to 8000 V 13,500 for Vh; step and hold at 8,000 V for 12,200 Vh. After IEF, strips were equilibrated 2 × 15 minutes in 10 ml equilibration buffer (6M urea, 75mM Tris-HCl pH 8.8, 29.3% glycerol, 2% SDS, 0.002% bromophenol blue) first containing 65 mM DTT, followed by equilibration buffer containing 135 mM iodoacetamide. Strips were briefly washed with SDS-PAGE running buffer (25 mM Tris base, 192 mM glycine, 0.1% SDS) and positioned on top of vertical 12.5% polyacrylamide gels containing 0.1% SDS. Strips were sealed into place with 0.5% agarose solution in SDS-PAGE running buffer. Gels were run in an Ettan DALT*six* electrophoresis system (GE Healthcare) at 20 W/gel with cooling at 10°C.

### *Protein visualization*

After electrophoresis, proteins were visualized by silver staining. Briefly, the proteins in the gels were

first fixed in 50% methanol, 12% acetic acid, and 0.05% formaldehyde for two hours, followed by two washes of 20 minutes in 35% Ethanol. After two minutes incubation in 0.02% sodium thiosulfate, gels were washed twice in $ddH_2O$ and incubated for 20 minutes in 0.2% silver nitrate solution. Gels were briefly washed for one minute, in $ddH_2O$, followed by development in 6% Sodium carbonate, and 0.02% sodium thiosulfate. Development was stopped with a 1.46% EDTA solution and gels were stored in 0.1% acetic acid at 4°C.

***In-gel digestion***

After silver staining, protein profiles on a 2D-Gel were analyzed using Image 2D Platinum software version 5 and the protein spots were picked up for further identification by mass spectrometry. Gel plugs were incubated in 3% $H_2O_2$ and dehydrated using acetonitrile, a reduction/alkylation process was performed by addition of 10 mM DTT followed by 100 mM iodoacetamide solution. The tryptic digestion was conducted overnight at 37°C. The peptides were extracted from gel plugs by incubation in 50% acetonitrile/0.1% TFA at room temperature for 20 min. The peptides were dried at 40°C and stored at –80°C. Before LC-MS analysis, peptides were resuspended in 0.1% formic acid.

***Protein analysis by mass spectrometry and Mascot software***

Nanoscale LC separation of tryptic peptides was performed with a NanoAcquity system (Waters Corp, Milford, MA) equipped with a Symmetry $C_{18}$ 5 µm, 180 µm 200 µm Trap column and a BEH130 $C_{18}$ 1.7 µm, 100 µm × 100 µm analytical reversed-phase column (Waters Corp). The samples were initially transferred to the trap column using an aqueous 0.1% formic acid solution at a flow rate of 3 µl/min for 3 min. Mobile phase A was water with 0.1% formic acid, and mobile phase B was 0.1% formic acid in acetonitrile. The peptides were separated using a gradient of 2% to 40% mobile phase B over 15 minutes at a flow rate of 1 µl/minute followed by a 3-minute rinse with 80% of mobile phase B. The column temperature was maintained at 35°C. The lock mass was delivered from the auxiliary pump of the Nano Acquity pump with a constant flow rate of 250 µl/min at a concentration of 200 fmol/µl of [Glu$^1$] fibrinopeptide B to the reference sprayer of the NanoLockSpray source of the mass spectrometer. Analysis of tryptic peptides was performed using a SYNAPT HDMS mass spectrometer (Waters Corp). For all measurements, the mass spectrometer was operated in the V-mode of analysis with a resolution of at least 10,000 full-width half-maximum.

All analyses were performed using a positive nanoelectrospray ion mode. The time-of-flight analyzer of the mass spectrometer was externally calibrated with [Glu$^1$]fibrinopeptide B from m/z 100 to 1600 with acquisition lock mass corrected using the monoisotopic mass of the doubly charged precursor of [Glu$^1$] fibrinopeptide B. The reference sprayer was sampled with a frequency of 30 seconds. Accurate mass LC-MS data were acquired with data direct acquisition mode. The spectral acquisition time was 0.5 second. In data direct acquisition mode, energy of trap was set at constant collision energy of 6 V. In transfer collision, control energy was set at 4 V. The quadruple mass analyzer was adjusted so that ions from m/z 400 to 1600 were efficiently transmitted.

***Data processing and protein identification***

LC-MS data were processed and searched using ProteinLynx GlobalServer software version 2 (Waters Corp). Protein identification was obtained with the embedded ion accounting algorithm of the software and searching a human database. The data were automatically mass measured, background subtracted and deconvoluted (charge state reduced). Peptide mass was corrected (based on the reference scans) by using Apex3D in data preparation tool. The processed data were compared with human protein database from NCBI and setting value of minimum peptide per protein was 2.

## Results and discussion

***Cytopathogenesis of SK-N-MC***

The coculture of SK-N-MC monolayers with *N. fowleri* trophozoite showed cytopathogenesis of the parasite. As soon as the parasites were added to the culture, they showed active progression and directional movement toward the host cells with hemispherical pseudopodia. Cytopathogenesis was evident by the presence of many floating target calls and the abundance of the amoebae. Accumulation of *N. fowleri* around the target cells and their engulfment was observed within 3 h post exposure (**Figure 1**). Moreover, the parasites accumulated around the host cells, and the morphology of infected SK-N-MC cells changed from a fibroblastic to a round shape.

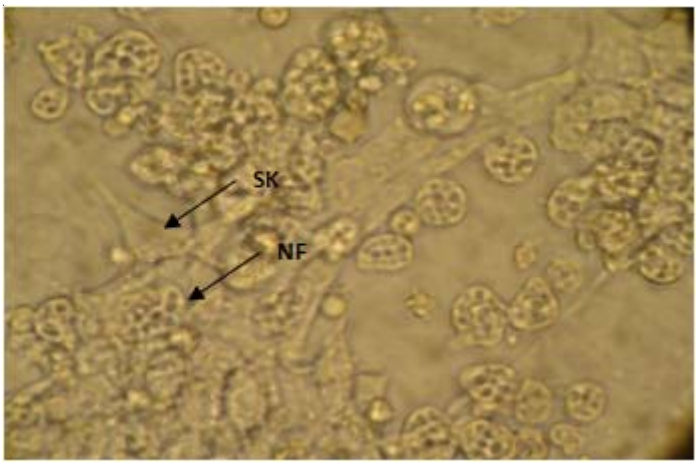


**Figure 1.** Co-culture of *Naegleria fowleri* (NF) trophozoites and human neuroblastoma SK-N-MC (SK) monolayer cells at 3 h showed a number of amoebae accumulated, attached, and engulfed SK-N-MC cells. Inverted microscope ×400

***Identification of N. fowleri trophozoite stress-responsive proteins by 2D-PAGE and LC-MS***

Comparative proteomic analysis was used to investigate the changes of protein profiles after the coculture of SK-N-MC cells with *N. fowleri* trophozoites for 0, 0.25, 1, 2, 3, 4, 5, and 6 hours. The SK-N-MC cells from the control and the treated group were harvested. Total protein was extracted and separated by 2D-PAGE. After image analysis, more than 60 protein spots were reproducibly detected and matched between all the gels. Proteins were well separated in both dimensions. The isoelectric points (pI) of the spots ranged from 4 to 7 and the molecular mass ranged from 14 to 100 kDa. Protein spots from all gels were compared and quantified using the ImageMaster 2D Platinum software version 5.0 (GE Healthcare). The identified protein spots corresponded to 53 proteins unique to human. It was noted that 24 of these proteins, identified in all noninfected SK-N-MC samples, could be classified into groups: signal transduction, transcription, translation, immune response, cytoskeleton, and stress response (**Table 1**). Under the same conditions, the results from the infected SK-N-MC showed those involved in fatty acid metabolisms, angiogenesis, signal transduction, transport, transcription, and translation (**Table 2**).

**Table 1.** Protein identities of noninfected SK-N-MC cells verified by 2D-PAGE at 6 hours

| Accession no. | Protein name | Function |
|---|---|---|
| gi\|15823651 | ALS2CR11 | Unknown |
| gi\|119583243 | hCG1784572 | Unknown |
| gi\|12232485 | interleukin-25 isoform 1 precursor | Signal transduction |
| gi\|119615716 | FLJ16008 protein, isoform CRA_b | Signal transduction |
| gi\|386854 | keratin subunit protein, type II | Cytoskeleton |
| gi\|50400889 | olfactomedin-like protein 1 | Transcription |
| gi\|12214173 | cytoplasmatic protein | Translation |
| gi\|73970880 | eukaryotic translation termination factor 1 isoform 2 | Translation |
| gi\|7767016 | lysozyme | Response to stress |
| gi\|2654381 | beta chain HLA-DQ molecule | Immune response |

**Table 2.** Protein identities of infected SK-N-MC cells verified by 2D-PAGE after exposure to *N. fowleri* for 6 hours

| Accession no. | Protein name | Function |
|---|---|---|
| gi\|7959923 | PRO2738 | Unknown |
| gi\|33338088 | MSTP132 | Unknown |
| gi\|119577941 | hCG2045077 | Unknown |
| i\|10719900 | brain-specific angiogenesis inhibitor 1 | Angiogenesis |
| gi\|7331218 | keratin 1 | Cytoskeleton |
| gi\|181402 | epidermal cytokeratin 2 | Signal transduction |
| gi\|2262235 | MAP kinase 7 | Signal transduction |
| gi\|4505587 | platelet-activating factor acetylhydrolase IB subunit gamma | Signal transduction |
| gi\|5410300 | COP9 complex subunit 4 | Signal transduction |
| gi\|21759799 | coiled-coil domain containing 87 | Signal transduction |
| gi\|20137773 | nuclear transcription factor Y subunit gamma | Transcription |
| gi\|34878757 | F-box only protein 25 isoform 3 | Transcription |
| gi\|55959248 | CREB regulated transcription coactivator 2 | Transcription |
| gi\|337516 | ribosomal protein s6 | Translation |
| gi\|16304168 | calcium channel beta 2c subunit | Transport |
| gi\|14043093 | EMILIN1 protein | Translation |
| gi\|148744384 | GALNT5 protein | Translation |
| gi\|33518888 | MORC-like protein | Translation |
| gi\|119621106 | hydroxyacyl-Coenzyme A dehydrogenase | Fatty acid metabolism |

### *Alteration of protein abundance after exposure to N. fowleri*

Only the protein spots from the 2D-PAGE analysis that exhibited reproducible and significant changes (>1.5 fold and $p < 0.05$) among the treatments were included for further analysis by LC-MS. All the protein spots were successfully identified by MS and ProteinLynx database searching with high confidence. Although the LC-MS evaluation showed similar results to the 2D-PAGE results, a number of proteins involved in the stress response, signal transduction and its pathways, and respiration were greatly affected (**Table 3**). In addition, the data from proteomic analysis showed that the presence of selected proteins produced in the SK-N-MC cells was altered after cocultivating with *N. fowleri* as early as 15 minutes postexposure and these changes remained throughout the study (**Table 4**).

Proteomics is a useful technique by which to study proteins specific to a given biological system and to understand interactions and relationships within a system. 2D-PAGE is one of the most used techniques that combines IEF (as a first dimension) and SDS-PAGE (as a second dimension) methods to separate the proteome at high resolution. Tiewcharoen et al. [5] demonstrated that the SK-N.MC cells are a suitable experimental system by which to study the cytopathogenesis of *N. fowleri*. According to PAM caused by *N. fowleri*, the protein alteration of infected human neuron cells is important for understanding the cytopathogenesis of *N. fowleri* infection during its invasion into its host cells.

In particular, our study used a GeLC-MS approach to identify protein alteration in SK-N-MC cells cocultured with the *N. fowleri* Siriraj strain to study the cellular interactions between a virulent amoebae *Naegleria.* strain and mammalian target cells *in vitro*. Identification of differentially expressed proteins by GeLC-MS/MS revealed the abundance of the proteins and we could infer the physiological changes of the SK-N-MC cells after the coculture.

Directed proteomics has provided information regarding novel biomarkers and possible therapeutic targets. We found that the abundance of cellular proteins involved in three cell processes: signal transduction, transcription, and stress response, of the SK-N-MC cells was significantly changed after the exposure to *N. fowleri* and oxidative stress-related changes in infected SH-SY5Y cellular proteins [11]. However, the heat shock cognate 71 kDa protein isoform 1 was found in infected SK-N-MC, while complex of heat shock protein 90 was detected in infected SH-SY5Y [11].

**Table 3.** Proteins of infected SK-N-MC cells identified by LC-MS that were changed in their abundance after coculture with *N. fowleri* for 6 hours

| Accession no. | Protein name | Function |
|---|---|---|
| gi\|292059 | MTHSP75 | Unknown |
| gi\|6650826 | PRO2044 | Unknown |
| gi\|220702506 | TapasinERP57 | Unknown |
| gi\|181402 | epidermal cytokeratin 2 | Signal transduction |
| gi\|2439517 | RHO/RAC effector protein | Signal transduction |
| gi\|5410300 | COP9 complex subunit 4 | Signal transduction |
| gi\|147640896 | coiled-coil domain-containing protein 88C | Signal transduction |
| gi\|24286012 | G-protein coupled receptor GPR115 | Signal pathway |
| gi\|28336 | beta-actin | Cytoskeleton |
| gi\|57471647 | vimentin | Cytoskeleton |
| gi\|35068 | Nm23 protein | Transcription |
| gi\|296736 | macropain subunit iota | Transcription |
| gi\|339647 | thyroid hormone binding protein precursor | Transcription |
| gi\|435476 | cytokeratin 9 | Transcription |
| gi\|4505773 | prohibitin | Transcription |
| gi\|5031753 | heterogeneous nuclear ribonucleoprotein H | Transcription |
| gi\|119626083 | albumin, isoform CRA_t | Transcription |
| gi\|515634 | ubiquinol-cytochrome c reductase core I protein | Respiration |
| gi\|303618 | phospholipase C-alpha | Phospholipid metabolism |
| gi\|438069 | thiol-specific antioxidant protein | Response to stress |
| gi\|5729877 | heat shock cognate 71 kDa protein isoform 1 | Response to stress |
| gi\|6470150 | BiP protein | Response to stress |
| gi\|7767016 | lysozyme | Response to stress |
| gi\|159162689 | protein disulfide isomerase | Response to stress |

Quantitative analysis of more than 100 proteins on 2D gels showed significant variations of 54 protein spots from SK-N-MC cells, which were confidently identified with MS (**Table 4**). Most of these proteins were assigned functions in human cells by Gene Ontology Categorizer (http://eagl.unige.ch/GOCat/). The majority of the proteins are involved in signal transduction and transcription. In addition, proteins implicated in translation, immune response, and transport were identified. For example, within the first 15 minutes after infection, the production of interleukin-25, which is involved in cell adhesion [8] and immune response [12], was triggered. Moreover, keratin may be produced to provide protection during the infection as a part of the host defense mechanism [13]. By contrast, a great number of proteins disappeared after exposure to the parasite. The disappearance was observed as early as 15 minutes postexposure. It is interesting that certain proteins that take part in the microstructure of a cell, such as EMILIN1, were also inhibited. This implies that the *N. fowleri* could induce the transformation of host cell morphology to facilitate the invasion of the parasite upon infection [14]. In addition, the parasite may take over the control of the infected host cell by regulating its signaling pathway, as the disappearance of COP9 was evident [15].

## Conclusions

Proteomic studies of infected neuroblastoma SK-N-MC monolayer cells post *N. fowleri* inoculation was demonstrated changes in their function, which were involved mostly in signal transduction and transcription including translation. This study contributes to knowledge of the sophisticated and finely-tuned molecular networks of the host cellular response to *N. fowleri*.

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**Table 4.** Detection of proteins from SK-N-MC cells after coculture with *N. fowleri* for 0.25, 1, 2, 3, 4, 5, and 6 hours

| Accession no. | Matched protein | Organism | Without *N. fowleri* Detection at time (hours) | | | | | | | With *N. fowleri* Detection at time (hours) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.25 | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 | 0.25 | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| gi\|119583243 | hCG1784572 | *Homo sapiens* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| gi\|119615716 | FLJ16008 protein, isoform CRA_b | *Homo sapiens* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| gi\|12214173 | cytoplasmatic protein | *Homo sapiens* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| gi\|12232485 | interleukin-25 isoform 1 precursor | *Homo sapiens* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| gi\|15823651 | ALS2CR11 | *Homo sapiens* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| gi\|2654381 | beta chain HLA-DQ molecule | *Homo sapiens* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| gi\|386854 | type II keratin subunit protein | *Homo sapiens* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| gi\|50400889 | olfactomedin-like protein 1 | *Homo sapiens* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| gi\|73970880 | eukaryotic translation termination factor 1 isoform 2 | *Canis familiaris* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| gi\|7767016 | lysozyme | *Homo sapiens* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| gi\|10719900 | brain-specific angiogenesis inhibitor 1 | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|119577941 | hCG2045077 | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|119621106 | hydroxyacyl-Coenzyme A dehydrogenase | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|14043093 | EMILIN1 protein | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|148744384 | GALNT5 protein | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|16304168 | calcium channel beta 2c subunit | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|181402 | epidermal cytokeratin 2 | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|20137773 | nuclear transcription factor Y subunit gamma | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|21759799 | coiled-coil domain containing 87 | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|2262235 | MAP kinase 7 | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|33338088 | MSTP132 | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|33518888 | MORC-like protein | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|337516 | ribosomal protein s6 | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|34878757 | F-box only protein 25 isoform 3 | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|4505587 | platelet-activating factor acetylhydrolase IB subunit d | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|5410300 | COP9 complex subunit 4 | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|55959248 | CREB regulated transcription coactivator 2 | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|7331218 | keratin 1 | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| gi\|7959923 | PRO2738 | *Homo sapiens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Score “1” = the protein was present and detectable, whereas score “0” means that the protein disappeared and became undetectable

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