Proteomic study of neuron and astrocyte cultures from senescence accelerated mouse SAMP8

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Abbreviations:

SAMP8, senescence-accelerated prone mouse strain 8

SAMR1, senescence-accelerated resistant mouse strain 1

Cox, cytochrome oxidase

AD, Alzheimer's disease

PP1, serine/threonine-protein phosphatase Type 1

PP2A, serine/threonine-protein phosphatase Type 2A

Ppp1ca, Serine/threonine-protein phosphatase PP1-alpha catalytic subunit

SUMO, small ubiquitin-like modifier

Aldh2, aldehyde dehydrogenase mitochondrial

GST, glutathione S-transferase

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Abstract

Senescent-accelerated prone (SAMP) strain 8 mice suffer an earlier development of cognitive aging-related pathologies and a shorter life span than conventional mice. Protein alterations in astrocytes, in addition to those in neurons, may contribute to neurodegenerative damage. We used proteomics to study cell-specific early markers of brain aging-related degeneration in SAMP8. The 2-D protein expression patterns of SAMP8 neuron and astrocyte cultures were compared to those obtained from the senescence-resistant strain 1 (SAMR1) cultures. Differentially expressed proteins identified by MALDI-TOF MS in both cell types belonged to cell pathways of energy metabolism, biosynthesis, cell transduction and signaling, stress response and the maintenance of the cytoskeletal functions. Most changes detected were cell type specific, but there was a general trend of increase of cell transduction, signaling and stress-related proteins and decrease of cytoskeletal proteins. Also, neurons showed an increase of the expression of several proteins involved in biosynthetic pathways. As a whole, the alterations of their proteoma indicated that both cell types are involved in the brain degenerative changes of SAMP8 mice, but network analysis suggests that neuronal changes are more complex and influencing. Cell culture proteomics is a powerful tool to discern cell specific changes and contributions to aging-related neuropathologies.

1. Introduction

Aging-associated neurodegeneration is the subject of an intense research in a hope for decreasing the incidence of mentally disabling diseases in the elder population. Alzheimer's disease (AD) is the most common aged-related neurodegenerative disease with 26 million of estimated people living with the condition worldwide and this number may quadruplicate by 2050.

The senescence-accelerated prone mouse strain 8 (SAMP8) is an established animal model to study aging-related cognitive decline, including the contribution of amyloid beta neuropathology [1,2] SAMP8 was obtained through phenotypic selection from a common genetic pool of AKR/J mice. It has a shorter life span than the reference strain senescence-accelerated resistant mouse strain 1 (SAMR1) and suffers aging-related cognitive impairment [3]. Brain studies of SAMP8 have shown elevated levels of amyloid beta [4] with plaque-like formation [5], thauopathy alterations [6] and oxidative stress [7,8,9] mainly attributed to mitochondrial disfunction [10]. Several authors have undertaken proteomic studies of SAMP8 brain tissue in a search for abnormal expression or oxidation of proteins that shed light on the pathologically altered pathways [2,11,12]. Although significant insights have been obtained, all key protein alterations underlying SAMP8 brain changes are far from been elucidated. We recently demonstrated that SAMP8 cultured astrocytes exhibited aging-related disturbances and a reduced neuroprotective capacity as compared to SAMR1 astrocytes [13], whereas studies on SAMP8 cultured neurons confirmed a diminished mitochondrial functionality (unpublished results). In this work, we further used the proteomics powerful technology to analyze SAMP8 brain protein expression changes down to the cellular level to dissect neuron and astrocyte contribution to brain pathological aging. For that purpose we used cultures of cerebral cortical neurons and astrocytes of SAMP8 and SAMR1 mouse and analyzed the differential changes appearing in the neuronal and astrocytic proteoma of SAMP8 as compared to SAMR1.

2. Material and methods

2.1. Animals and reagents

SAMR1 and SAMP8 mouse breeders were purchased from Charles River Laboratories (Lyon, France) and their colonies were established in the Animal House of the Universitat de Barcelona. Animals were maintained and handled in compliance with protocols approved by the Generalitat de Catalunya, Spain, in accordance with EU guidelines, and in compliance with the Office of Laboratory Animal Welfare/National Institute of Health (identification number A5224-01). Culture chemicals were purchased to Sigma-Aldrich Co (St. Louis, MO, USA) if not otherwise stated. Reagents and analytical grade chemicals for electrophoresis and gel staining were obtained from Merck (Darmstadt, Germany) or as indicated below.

2.2. Cell cultures

Neuron cultures

Primary cultures of neurons were prepared from the fetal cerebral cortices of E15-E18 SAMR1 and SAMP8 mice as previously described [14]. Neocortices were dissected and mechanically minced. Tissue was then dissociated to single cells by mild trypsinization (0.02% w/v) at 37°C for 10 min followed by trituration in a DNase solution (0.004%-w/v) containing a soybean trypsin inhibitor (0.05%-w/v). The cells were re-suspended in a DMEM (Biochrom AG, Berlin, Germany) supplemented with 100 mU/L insulin, 7 µM p-aminobenzoic acid, 0.2 mM glutamine, 100 µg/mL gentamicin and 10% FBS (Gibco-Invitrogen, Paisley, UK). The cell suspension was seeded at 32×10^4 cell/cm² in T75 flasks (Nunc, Roskilde, Denmark) pre-coated with poly-D-lysine, and incubated for 7-8 days in a humidified 5% CO₂/95% air atmosphere at 37 °C. A mixture of 5 µM 5-fluoro-2'-deoxyuridine and 20 µM uridine was added after 48 h in culture to prevent glial proliferation. Neurons were collected after 7 days in vitro.

Astrocyte cultures

Primary cultures of astrocytes were established from cerebral cortical tissue of 2-day old SAMR1 and SAMP8 as previously described [13]. Briefly, cortices were dissected free of the meninges, diced into small cubes and dissociated by incubation with a 0.25 % trypsin / 1 mM EDTA solution (Gibco) for 25 min. After a further mechanical disaggregation, cells were resuspended in DMEM supplemented with 2.5 mM glutamine, 100 µg/mL gentamycin and 20% FBS. Cells were seeded at 5×10^4 cell/cm² in T75 flasks and incubated at 37°C in a humidified atmosphere of 5% CO₂ - 95% air. The culture medium was changed twice a week. The concentration of FBS was changed to 15% and 10% after one and two weeks of culture, respectively. After 3 weeks, the flasks were shaken in an orbital shaker at 200 rpm for 4 h to dislodge microglia and the attached astrocyte monolayer was collected.

2.3 Two-dimensional gel electrophoresis

Sample preparation

The neuron or astrocyte monolayer of a culture flask was washed with cold PBS and maintained on ice while the cells were scraped with a rubber policeman. Cells were aseptically collected, centrifuged at 400 x g, rinsed with PBS and the pellets stored at -80°C until processed. Three different flasks from each of three independent cultures of SAMR1 and SAMP8 were harvested for each cell type.

Frozen samples were resuspended in 500µl of cold lysis buffer (7 M urea, 2 M thiourea, 4 % CHAPS (w/v), 40 mM Tris-base, 10 mM DTT and 1 mM PMSF), sonicated for 30 s, shaken orbitally for 10 min, sonicated 30 s again and centrifuged at 15000 ×g for 10 min, all at 4°C. Total proteins were precipitated from the supernatant by orbital shaking in 10 % trichloroacetic acid for 90 min at 4°C and centrifugation at 15000 ×g for 10 min. The proteins extracts were rinsed with iced acetone, resuspended in 500µl of rehydration buffer (7 M urea, 2 M thiourea, 2% CHAPS (w/v), 12µl/ml DeStreak, 0.5 % IPG Buffer), and stored in aliquots at -80°C. Protein concentration was determined using RC DC Protein Assay kit (Bio-Rad, Hercules, CA, USA), according to manufacturer instructions.

Isoelectric focusing

For the first dimension, a 24-cm (neurons) or 18-cm (astrocytes) pH 3-10 IPG strip (GE Healthcare, Chalford St. Giles, Buckinghamshire, UK) was loaded at 20°C for 6

h with 100 µg of protein solubilized in 350 µl - 450 µl of rehydration buffer. The strips were covered with IPG Cover Fluid. Electrophoresis was performed at 20°C using an Ettan IPGphor unit, IPGphor apparatus (Amersham Biosciences, Piscataway, NJ, USA) under the following conditions: 50 V (fixed for 6h), 500 V (gradient over 1h), 1000 V (gradient 1h), 4000 V (gradient 1h), 8000 V (gradient 1h) and 8000 V (fixed to 60000 1h). The electrofocused strips were stored at -80°C until SDS-PAGE electrophoresis.

SDS-PAGE

Previously to the second dimension electrophoresis, the IPG gel strips were reduced for 15 min in SDS equilibration buffer (50 mM Tris-HCl pH 8.8, 6 M urea, 30% glycerol (w/v), 2% SDS (w/v), and a trace of bromophenol blue) containing 64.5 mM DTT. This was followed by a 15 min wash in alkylation buffer containing 135.1 mM iodoacetamine. SDS-PAGE was carried out on 12% polyacrilamide gels with 30% Duracryl, 0.8% Bis (Genomic Solutions, Ann Arbor, MI, USA) at a voltage of 2.5 W for 30 min and 12.5 W for approximately 5h, using the Ettan Dalt VI system (Amersham Biosciences). The equilibrated strips were embedded on top of the gels using 0.5% w/v agarose sealing solution with a trace of bromophenol blue. The running buffer was 2.5mM Tris-base, 19.2 mM glycine, 0.1% SDS. The 2D gels were fixed in 40% ethanol, 10% acetic acid for 30 min and the spots were visualized by silver staining. The molecular mass of the proteins was determined by running standard protein markers (Bio-Rad Laboratories, Hercules, CA) covering the range of 10-250 kDa. The *pI* values were given by the strips supplier.

2.4 Image analysis and statistics

The 2D gels were discolored with water, scanned using a densitometer (GS-800, Bio-Rad) and analyzed with ImageMasterTM 2D Platinum 5.0 software (Amersham Biosciences). A total of nine gels for each cell type and mouse strain were analyzed. Neuron and astrocyte gels were analyzed separately. A reference gel was constructed for each cell type using as a basis the gel with the maximum number of distinct spots. All the spots for a given gel were matched to the corresponding reference gel using the automatic analyses and were verified manually by local pattern comparison. Quantification of proteins was expressed as relative volumes of all spots in each gel (% volume). The protein spots of SAMP8 mice were accepted as different from SAMR1 mice when the protein content was 50% increased or decreased and the Student's *t*-test confirmed statistical significance of the results ($p < 0.05$).

2.5 MS protein identification

The spots of interest were excised and subjected to in-gel digestion with trypsin as described elsewhere [15]. Triptic peptides were extracted from the gel with acetonitrile/water/trifluoroacetylacetone, and extracts were evaporated to dryness and redissolved in acetonitrile/water (1/1), 1% acetic acid. Protein identification was carried out by means of peptide mass fingerprinting using a Voyager DE-PRO MALDI-TOF MS (Applied Biosystems, Foster City, CA). A small aliquot (0.5 mL) of the tryptic extract was mixed with 0.5 mL of α -cyano-4-hydroxycinnamic acid (5 mg/mL). External calibration was carried out with a set of synthetic peptides (Applied

Biosystems). The Protein Prospector (<http://donatello.ucsf.edu>) and MASCOT (Matrix Science, Inc, Boston, MA) software packages were used for data mining in the Swiss-Prot (European Bioinformatics Institute, Heidelberg, Germany) and National Center for Biotechnology Information (Bethesda, MD) databases.

2.6 Network analysis

A network analysis was performed with the Combinatorica and Graph Utilities Packages of Mathematica, version 6 (Wolfram Research, Inc., Champaign, IL) for both groups of differential proteins, neuronal and astrocytic. Protein-protein interaction was obtained from the Human Protein Reference Database (<http://www.hprd.org>) [16]. Interactome network (HPI) is build with 34876 interactions between 9047 proteins, that corresponds to the largest connected component from the original network with 35021 interactions between 9462 proteins.

2.7 Western Blotting

One representative protein for each cell type were selected to validate the proteomics results by Western blotting. Cultured cells were lysed for 10 min on ice in RIPA buffer containing a protease inhibitor cocktail (Complete, Roche, Basel, Switzerland) and 1 mM orthovanadate. They were collected, sonicated to further lyse the cells, centrifuged and the supernatants frozen at -20°C until assay. Proteins were quantified by the Bradford method and 15 μg of protein extracts were denatured, loaded onto a SDS-polyacrylamide gel and electrophoresed. Proteins were transferred onto PVDF membranes (Immobilon-P, Millipore, Bedford, MA). Neuron membranes were incubated with anti-PP1-alpha catalytic subunit (Ppp1ca) (Cell Signaling, Danvers, MA) and astrocyte ones with anti-Aldehyde dehydrogenase (Aldh2) (Santa Cruz Biotechnology, Santa Cruz, CA), diluted **1:1000**. Membranes were then incubated with horseradish peroxidase conjugated secondary antibodies. Proteins were detected with a chemiluminescence detection system based on the luminol reaction. Densitometric analysis of the digitalized immunoreactive bands was performed using Quantity One software (Bio Rad). The levels of protein immunoreactivity were normalized to that of GAPDH by incubation with anti-GAPDH (Assay Designs, Ann Harbor, MI) **1:1000**.

3. Results

The 2D gels of cultured SAMR1 and SAMP8 cerebral cortical neurons and astrocytes yielded a range of 700-1200 spots per gel. Fig 1 shows representative 2D gels after silver staining. The proteomic analysis of the neurons of the two mouse strains showed 21 differential protein spots in SAMP8 as compared to SAMR1, which are listed in Table 1. Three additional spots were not identified (not shown). A total of 18 differentially expressed proteins were obtained in astrocytes of SAMP8 as compared to SAMR1 mice. All were identified, as listed in Table 2. MS data for each spot are

summarized in Table 3 and 4 for neurons and astrocytes, respectively. All the mass spectra (not shown) of the peptides from the identified proteins were matched to the mass spectra of protein databases with a high probability-related Mowse score, whereas Mascot score was instead used for one mass spectrum (see Table 3).

Multiple differential spots were detected for two neuron proteins, tubulin alpha-1A chain and tubulin beta-5 chain. This indicated a differential expression in several coexisting posttranslational modifications of these proteins. Identified neuron and astrocyte proteins can functionally be classified as those involved in the energetic metabolism of the cell, biosynthetic pathways, transduction and signaling mechanisms, response to cellular stress and cytoskeletal functions. Additionally, one identified astrocyte protein has a transport function and a neuronal protein has an unknown function in mammals.

Densitometric analysis and representative images of western blot are shown in Fig 2. Significant increases in a signaling protein in neurons (Ppp1ca) and in a stress response protein in astrocytes (Aldh2) were in agreement with the proteomic analyses. Network analyses were performed with 17 neuron and 10 astrocyte differentially expressed proteins that were connected to other proteins in the HPI (Table 5, Fig 3). Analytical parameters showed that neuron proteins were closely related between them because they were connected through a shorter distance than average in HPI. Also, a much higher centrality parameters than the whole HPI indicates that these neuron proteins participate in many relevant cell pathways. Astrocyte protein network showed an increase in two centrality parameters that indicate its inclusion in more cell pathways than average in HPI.

4. Discussion

The proteomic study of neuron and astrocyte cultures of SAMP8 mouse showed a differential pattern of protein expression alterations as compared to those of the reference strain SAMR1. The function of involved proteins and the possible significance of the changes are discussed below.

Energy metabolism.

A decrease in acyl coenzyme A thioesterase 1 expression was found in neurons and astrocytes. Besides, there was an increase of a more acidic form of the precursor of acyl coenzyme A thioesterase 2 in both neurons and astrocytes. Acyl-coenzyme A thioesterases catalyze the hydrolysis of acyl-coenzyme A to the free fatty acids and coenzyme A, providing the potential to regulate intracellular levels of these molecules. Isoform 1 is cytoplasmic and isoform 2 is mitochondrial. Fatty acids and its activated form acyl-coenzyme A are key components of numerous energy metabolic processes of biosynthesis/catabolism of lipids. The enzyme phosphoglycerate mutase 1, that catalyzes the interconversion of 3-phosphoglycerate and 2-phosphoglycerate during glycolysis and gluconeogenesis, showed decreased expression in SAMP8 neuron proteoma. Decrease of this enzyme has been reported in the hippocampus proteoma of AD [17]. It plays an important role in maintaining synaptic function. Decrease of glycolysis has been related to the abnormal low levels of brain acetylcholine in aged SAMP8 [18]. SAMP8 astrocytes showed an increase of adenylate kinase isoenzyme 4, a phosphotransferase that catalyzes the interconversion of adenine nucleotides and therefore regulates mitochondrial respiration and cytosolic energy metabolism. Astrocyte cultures also showed a significant decrease of the

precursor form of cytochrome oxidase (Cox) isoform 4-1. Cox catalyzes the electron transfer from ferrocycytochrome *c* to oxygen in the mitochondrial electron transport chain. Isoform IV-1 predominates in brain tissue. Defective energy metabolism in aging and AD has been linked to decreased Cox 4 activity levels [19,20].

Biosynthesis.

SAMP8 neuron proteoma showed an increase of both enzymes that sequentially catalyzed L-serine biosynthesis: D-3-phosphoglycerate dehydrogenase and phosphoserine aminotransferase. L-serine is a building block for the synthesis of proteins and neural phospholipids. Also increased in SAMP8 neurons was ribose-phosphate pyrophosphokinase 1. Its product phosphoribosyl pyrophosphate is a substrate in the biosynthesis of histidine, tryptophan and purine and pyrimidine nucleotides. Astrocytes showed a decrease of dihydropteridine reductase, enzyme involved in the maintenance of tetrahydrobiopterin levels. The later is a cofactor in the synthesis of amino acid monooxygenases and nitric oxide production. SAMP8 astrocytes showed a more alkaline form of S-methyl-5-thioadenosine phosphorylase, a pentosyltransferase that participates in adenine and methionine metabolism and in the biosynthesis of polyamines.

Cell transduction and signaling.

SAMP8 neuron proteoma showed an increase of poly(rC)-binding protein 1 and protein 2, but only poly(rC)-binding protein 1 was increased in astrocytes. They are the two major poly(C)-binding proteins in mammalian cells and mediate a variety of post-transcriptional functions related to mRNA stability and expression. F3-like protein 1 was also increased in SAMP8 neurons. It is involved in neuron differentiation and in maintaining cell type-specific functions. It would act as a transcriptional repressor. Also increased were specific subunits of two key serine/threonine-protein phosphatases, known as Type 1 (PP1) and Type 2A (PP2A). Reversible protein phosphorylation is a fundamental mechanism by which many biological functions are regulated. PP1 participates in protein synthesis and long-term synaptic plasticity. It has been shown that PP1 needs to be inhibited to gate LTP, because it limits learning and favors forgetting [21]. Therefore, PP1 upregulation may contribute to the cognitive deficiencies in SAMP8 mice. Caspase-3 precursor was increased in SAMP8 neurons. This proenzyme undergoes proteolytic processing after a variety of apoptotic stimuli and then acts as effector caspase leading to cell apoptosis.

In astrocyte SAMP8 cultures there was an increase of the enzyme protein-arginine deiminase type 2, which catalyzes the conversion of arginine to citrulline. Citrulline is not incorporated into proteins, thus deimination of arginine residues appears to form a post-translational protein modification. Further, this enzyme may also be involved in nitric oxide synthesis. Also in astrocytes there was a decrease of adenosylhomocysteinase. It is a reversible enzyme that regulates the intracellular concentration of adenosylhomocysteine, a methyl donor for transmethylation involving proteins, lipids and nucleic acids. DNA methylation is critically important in mediating precise CNS gene regulation in response to proper environmental signals.

Stress response proteins

SAMP8 neuron proteoma showed an increase of erlin-2, also named SPFH domain-containing protein 2. It is a key component of recently described pathway for endoplasmic reticulum-associated degradation where it would act as a substrate recognition factor. This pathway accounts for the degradation of aberrant proteins and some others that are metabolically regulated [22]. Also increased is the small ubiquitin-like modifier (SUMO)-activating enzyme subunit 1, that integrates a nuclear and cytoplasmic dimeric enzyme mediating the ATP-dependent activation of SUMO proteins. It is the first enzyme in the pathway leading to formation of ubiquitin-protein conjugates and therefore it is rate-limiting in the conjugation and increases in response to oxidative stress. The related ubiquitin-like protein NEDD8 was reported overexpressed in aged SAMP8 [23]. More broadly, the ubiquitin/proteasoma pathway is critical for cell survival and repair and is involved in many cellular processes and aged-related diseases [24]. A marked increase of brain ubiquitin has been reported in AD [25].

In astrocytes there was an increase of sodium/hydrogen exchanger 5.

Sodium/hydrogen exchangers comprise a family of integral plasma or mitochondrial membrane proteins involved in a variety of physiological processes such as pH regulation, volume control and electrolyte transport. Isoform 5 is restricted almost exclusively to the brain. Its increase in SAMP8 could be linked to increased demand to eliminate acids generated by metabolism under pathophysiological conditions or to counter adverse environmental conditions.

Two antioxidant enzymes were increased or decreased, respectively, in the SAMP8 astrocyte proteoma. The enzyme Aldh2 is involved in the detoxification of reactive aldehydes in the mitochondria. An increase of Aldh2 has been described in the brain of old rats [26] in the olfactory system of aged mice [27] and in AD temporal cortex [28]. Glutathione S-transferase (GST) A4 was decreased in SAMP8 astrocytes. The alpha class of glutathione S-transferase (GST) enzymes, that includes GST A4, has a physiologically relevant activity against oxidative stress. GST is reported decreased in Down syndrome fetal brain [29], a genetical disorder linked with oxidative stress and AD-like pathology at the middle age.

Cytoskeletal proteins

Actin cytoplasmic 1 (beta-actin) was decreased in both SAMP8 neurons and astrocytes and actin cytoplasmic 2 (gamma-actin) was decreased only in neurons. They coexist in most cell types as components of the cytoskeleton and mediators of internal cell motility. Tubulin is the major constituent of microtubules and is formed by dimers of alpha and beta chains. The isotypes 1A and 3 of the alpha chain and the 5 isotype of the beta chain were decreased in SAMP8 neurons. The appearance of differential expression in a number of spots for both tubulins is in agreement with the reported high diversity of modifications in cytoskeletal proteins [30]. Disruption of microtubule-based transport and synaptic mechanisms occurs in AD neurons [31]. Decreased tubulin alpha and beta chains has been reported in aged rodent brain [32,33]. Finally, the two actin-related proteins alpha-centractin and macrophage capping protein presented reduced expression in the SAMP8 astrocyte proteoma. Alpha-centractin is a component of the dynactin complex. Macrophage capping protein, discovered in macrophages, is present in a variety of tissues where binds and caps actin filaments. Deficiency of either contractins or capping proteins may contribute to derangement of cellular organelle motility in SAMP8. Alpha-centractin and the actin-capping protein F have been described decreased in fetal Down syndrome brain [34]. The latter also was reduced in aged rat hippocampus [32]. This

decreased expression of specific skeleton protein is in general agreement with the cortical atrophy, axonal dystrophy with reduction of dendritic spines, and membrane and transport alterations that has been described in SAMP8 brains [35]. On the other hand, a few increased cytoskeletal proteins were fascin in neurons and stomatin-like protein 2 and myosin light polypeptide 6 in astrocytes. Fascin is an actin crosslinking protein that is present in brain. It organizes actin filaments into tightly packed bundles and contributes to the architecture and function of cell protrusions and microfilaments. Stomatin-like protein 2 is an unusual member of the cytoskeletal stomatin family. Recent studies have associated stomatin-like protein 2 with the mitochondrial membranes and the maintenance of mitochondrial morphology [36]. Myosin is composed of 2 heavy chains and 4 light chains where light polypeptide 6 is a regulatory chain. Myosin anchors to actin filaments and induces a movement coupled to the hydrolysis of a nucleoside triphosphate.

Miscellaneous

Coatomer subunit epsilon was decreased in the SAMP8 mouse astrocyte proteoma. The coatomer is a cytosolic protein complex formed by at least seven subunits. It associates with specific Golgi vesicles to mediate biosynthetic protein transport from the endoplasmic reticulum to the Golgi network. It also mediates retrograde transport of immature or abnormal proteins from Golgi to endoplasmic reticulum. Significance of WD repeat-containing protein 61 increase in the SAMP8 neuron proteoma can not be speculated by now. The WD-sequence repeat-containing family of proteins is a large family with a large diversity of roles in eukaryotic cells. The role of WD repeat protein 61, also known as Rec14, is not known in mammals. In yeast is functional in early stages of meiosis.

The proteins abnormally expressed in cultures of both neurons and astrocytes of SAMP8 were associated with pathways similar to those reported altered in brain tissue of SAMP8, aged brain or AD brain. These results showing a general agreement with previous proteomic studies reporting differential expression and elevated oxidation of proteins belonging mainly to energy metabolism, antioxidant defence, clearance of proteins, cell signaling and cytoskeletal integrity functions [2, 37, 38]. The proteomic study of neurons and astrocytes demonstrated that both cell types are implicated in the brain protein pathway alterations of this mouse model of aged related neurodegeneration. Accordingly to the network analysis, many proteins affected in neurons belonged to more crucial and complex pathways than those in astrocytes and its alteration will be highly disruptive for the cognitive processes. On the other hand, astrocyte protein alterations are in agreement with its reported neuroprotective capacity loss [13].

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5. Conflict of interest statement

The authors declare that there is not any financial or commercial conflict of interest.

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Figures

A

B

Fig 1. – Representative images of silver stained 2D gels of SAMR1 and SAMP8 neuron (A) and astrocyte cultures (B). Reference number of differential spots is indicated for both cell types. Gels are obtained as described in Methods.

Fig 2. – Representative immunoblots and densitometric analysis of selected proteins differentially expressed in SAMP8 and SAMR1 neuron or astrocyte cultures. Levels of Ppp1ca in neurons (A) and Aldh2 in astrocytes (B). * $p < 0.05$, Student's *t*-test.

Fig 3.- Neuron (A) and astrocyte (B) interacting protein networks for differentially expressed proteins in SAMP8 versus SAMR1. Connecting line thickness indicates proximity: 1, direct connection; 2, 3 and 4, connected through 1, 2 and 3 intermediate nodes, respectively; absence of line, connected through 4 intermediate nodes. Proteins are represented by their gene names, listed in Tables 2 and 4.