Heterogeneity of Cells Population and Secretome Profile of Differentiated Cells from E17 Rat Neural Progenitor Cells

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Abstract

Conditioned medium has now gained increasing interest since the development of secretome-based therapy. Various types of cells have been studied as a source of the secretome. One of them is neural progenitor cells (NPCs). These are cells that capable of differentiating into neurons as well as glial cells. Indeed, the study on NPCs has risen in the last few decades, but the study on the differentiated cells has not clearly described. The most common procedures that widely used to get the conditioned medium is starvation. However, cell starvation may cause environmental stress and become an apoptotic trigger for the cells. In this study, we analyzed the effect of starvation on differentiated cells from E17 rat neural progenitor cells (NPCs) based on cells characteristics and secretome profile. We found that starvation decreased cells viability and affected the heterogeneity of the cell population. Astrocytes survived more under nutrient deprivation conditions, and the progenitor cells showed a higher tendency to differentiate to glial cells than neurons. Duration of starvation also influenced the secretome profile, alterations found in protein types and also their function in the biological process. During 24 hours of starvation, cells secreted proteins that were used to maintain cell growth, stimulate differentiation, and produce energy, but there were also proteins that identified and involved in autophagy activation. After 48 hours of starvation, astrocytes that became the dominant cells secreted proteins that try to keep protecting the remaining neurons.

Key Words: Conditioned medium; Differentiated cells; Neuron; Astrocytes; Secretome

Introduction

The use of secretome gained increasing attention since many researchers believed the paracrine hypothesis in which injecting cytokines, chemokines, or growth factor can cause significant repair or regeneration of injured tissues compared to implanting cells directly to the organs. Secretome can be obtained from conditioned medium of specific cells that released desired proteins.

One of the promising sources of secretome and being extensively investigated is neural progenitor cells (NPCs). These are cells that are capable of differentiating into neurons as well as glial cells and has been reported as an ideal tool to investigate neural cell differentiation, in vitro testing of neuroactive drugs, modelling neural diseases, or explore effective future cell-based therapies[1,2]. Moreover, NPCs not only can be used as a vast source of neuronal cells but also can secrete several growth factors and cytokine which widely used as secretome therapies instead of cells therapies in regenerative medicine[3,4,5]. Secretome-based therapy from both NPCs or MSCs reduces disease severity in animal model diseases such as inflammatory arthritis, Parkinson’s disease, experimental traumatic brain injury, and myocardial infarction[6-3]. In vitro studies also reported that secretome enhances the proliferative and migratory abilities of various types of cells[8,9,10]. However, how then the NPCs differentiate and do the differentiated cells of NPCs secrete certain proteins has not clearly described.

There are several methods to produce conditioned medium that contained secretome and to decipher its protein contents[11,12]. Unfortunately, serum supplemented media which is the most common media used in cell culture will give analytical challenges because of the masking effect of highly abundant serum proteins that caused secreted proteins analysis more complicated and extremely difficult. Whereas, proteomics studies is very important to delineate molecules and pathways critical for NSC biology and can answer questions about how NPCs or the differentiated cells can participate in neural repair[13]. Hence, serum deprivation is needed to overcome that problem.

However, serum deprivation, or also named as cell starvation, can also cause environmental stress and become an apoptotic trigger for the cells[14]. These conditions have been reported to influence cellular phenotypic characteristics, induce a swift and dynamic response, elicit complex and unpredictable time-dependent effects, and the cell-type dependent effect can interfere with the experimental results[15].

In this study, we analyzed the effect of starvation on differentiated cells from E17 rat neural progenitor cells (NPCs) based on cells characteristics and secretome profile. We found that serum deprivation affected cells’ viability, the pattern or tendency of differentiation, and secretome profile. Noteworthy is that the duration of starvation was very influential for differentiated cells of NPCs under serum deprivation culture conditions.
Materials and Methods

Isolation and Culture of Differentiated Cells of Rat Neural Progenitor Cells (NPCs)

Wistar rat embryos (E17) were used in this study. Three replicated trials were carried out. The pregnant female rat was euthanized by intraperitoneal injection of ketamine-xylazine cocktail (91 mg/kg ketamine + 9.1 mg/kg xylazine) 0.2 ml/100 g body weight. Uterus was exposed by medial cutting in aseptic condition to avoid contamination. All fetuses were removed and stored in sterile dissection solution (HBSS containing 0.3% glucose).

Whole brains were isolated and dissected into small pieces for the further digestion process. Dissected brain tissues were centrifuged at 300xg for 2 minutes, and the supernatant was discarded. Digestion process was done using Neural Tissue Dissociation Kit (T) (Miltenyi Biotec) based on manufacturer protocols. Cells then were cultured in 24 wells 0.1 % gelatin coated dishes overnight at cell density (5x10⁴ cells/cm²). Medium used in this step was neurobasal medium (NM) MACS®-Neuro-Medium (Miltenyi Biotec) containing 2% MACS NeuroBrew-21 (Miltenyi-Biotec), 1% antibiotic-antimycotic (100x) (GibcoTM), 10% fetal bovine serum (FBS; GibcoTM) and 1% GlutaMax® (GibcoTM). The primary culture was done in 4 days and after that cells were characterized by flow cytometry and immunocytochemistry, being starved for 24 hours and 48 hours.

Experimental procedures of this research were approved by the Animal Care and Ethics Committee of National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia (No: LB02.01/2/KE.150/2017). Cells Starvation and Conditioned Medium Collection

After four days of primary culture, cells were cultured in serum-free condition by changing the medium with MEM medium (GibcoTM) without any supplement addition. After 24 hours, the conditioned medium (CM) was collected and changed with the fresh equilibrated medium. CM was collected again on the next 24 hours to identify secretomes which were secreted by differentiated cells from NPCs which had starved for 48 hours. Conditioned medium was collected from three replicates and then filtered using 0.22 µm size of the pore and further used for secretome analysis. Trypan blue exclusion test was used for cells viability assay.

Flowcytometry

Differentiated cells of NPC were characterized four days after culture before starvation treatment, 24 hours, and 48 hours starvation duration with PSANCAM-APC and A2B5-PE (Miltenyi Biotec). Staining steps were done following the instruction of the staining kit. Flowcytometry process and analysis were done using BD Accuri™ C6 Plus flow cytometer.

Immunocytochemistry

Besides being characterized by flow cytometry, cells were also characterized by immunocytochemistry with GFAP (Santa Cruz sc) and NeuN (Abcam ab104225) antibody markers. The secondary antibody used in this study was secondary HRP-conjugated antibody (Trekkie Universal Link, Starr Trek Universal HRP Detection Kit Bicore®). Staining procedures referred to the previous study by Rinendyaputri et al.[10].

Secretome Analysis

The conditioned medium, which contained secretome from the differentiated cells of NPCs, were analyzed by LC/MS-MS. Protein concentration was determined by Bradford's method following the protocol of the User Guide Coomassie (Bradford) Protein Assay Kit and data was analyzed by Thermo SkanIt RE for Multisize GO software version 3.2. Protein from conditioned medium (10 µg) proceeded for peptide preparation and in solution digestion using In-Solution Tryptic Digestion and Guanidination Kit (Thermo Scientific).

Peptide clean up was done using Pierce C18 Spin Column (Thermo Scientific), the cleaned sample was then dried in a vacuum concentrator for 2 hours. The dried sample was then dissolved in 50 µl dissolving solution, 2.5 µl (500 ng peptides) and then continued to be fractionated. Fractionation results were analyzed using QExactive Mass Spectrometry (Thermo Scientific). Output data from LC-MS/MS were analyzed using Proteome Discoverer software 2.1 (Thermo Scientific) to identify proteins based on the Rattus norvegicus database (TaxonomyID: 10116) to identify the type of proteins from the conditioned medium. Gene ontology analysis was done using the protein analysis through evolutionary relationship (PANTHER) classification system site (http://pantherdb.org).

Statistical Analysis

Data evaluation was performed using one-way ANOVA to compare the mean values for the groups. The significance value was set at p ≤ .05. The results are presented as mean ± SEM (standard error of the mean).

Results

Cells Heterogeneity of Differentiated Cells of NPCs

The expressions of surface markers of the differentiated cells of NPCs after four days culture was analyzed by flow cytometry using two markers, which are PSA-NCAM and A2B5. The results showed that there was heterogeneity of the differentiated cells that consisted of immature neuron PSA-NCAM⁺, glia progenitor A2B5⁺, and double positive of both markers (PSA-NCAM⁺/A2B5⁺) as shown in Figure 1. Cells with PSA-NCAM⁺ was the dominant cells in the population on the fourth day of the culture (61.0 ± 0.71 %), A2B5⁺ was 24.15 ± 1.77 %, while cells with double positive expression were 14.05 ± 2.62 %. The cells were then cultured in serum-free medium with low protein content for starvation treatment.

We found that the treatment altered heterogeneity profile of the cells culture after 48 hours of starvation. In detail, after 24 hours starvation, the percentage of two types of the cell population was decreased, but the alteration was not statistically significant, they were 52.6 ± 4.24 % for PSA-NCAM⁺ and 12.6 ± 0.29 % for PSA-NCAM⁺/A2B5⁺. On the other hand, A2B5⁺ cells were increased (33.55 ± 3.47 %), which still showed no significant difference. These patterns, the decrease of two types cells and the increase of A2B5⁺, were continued after 48 hours starvation. Cells with positive expression of A2B5 markers became the highest (89.75 ± 2.9 %) while PSA-NCAM⁺ greatly decreased (3.75 ± 1.2 %) similar with the expression of double positive cells (1.7 ± 0.71 %) (Figure 1).

Heterogeneity profile alteration correlated with the viability of the cells. Duration of starvation for 24 hours decreased cells viability from 91.65 ± 1.28 % at four days culture into 90.5 ± 0.3 %. The viability of the cells continued to decline with starvation duration; after 48 hours, starved cells viability was 83.82 ± 0.89 % (Figure 2).

Cells were stained with NeuN and GFAP to analyze differentiation that may cause heterogeneity profile of cultured cells, presented in Figure 3. Quantitatively, the results showed that after being isolated and cultured for 4 days, differentiation to be mature neuron (NeuN positive) or glial cells (GFAP cells) were 18.95±4.78 for NeuN and 17.83±5.34 for GFAP. GFAP positive cells were significantly increased after 48 hours starvation (52.41±4.25) (Table 1).
Cells heterogeneity and secretome of differentiated cells from NPCs

Figure 1: Heterogeneity profile of Differentiated Cells of E17 Rat Neural Progenitor cells on day 4, 24 h and 48h after serum deprivation; (A) Percentage of PSA-NCAM+, A2B5+, and double positive expression of both markers, presented as mean ± SEM calculated from three replicates; (B) Flowcytometry results X axis : PSANCAM-APC, Y axis : A2B5-PE (cell population; isotype; cells immunophenotyping) on day 4 (1), 24 h after serum deprivation (2), and 48 h after serum deprivation.
**Figure 2**: Viability of Differentiated Cells of E17 Rat Neural Progenitor Cells on Day 4, 24 Hours and 48 Hours After Serum Deprivation Culture

**Figure 3**: Immunocytochemistry of Differentiated Cells of E17 Rat Neural Progenitor Cells using NeuN and GFAP markers on Day 4 culture (A-C), 24 hours (D-F) and 48 hours After Serum Deprivation Culture (G-I); negative control (A,D,G), NeuN Staining (B,E,H); GFAP Staining (C,F,I). Scale bars = 25 µm.
**Cells heterogeneity and secretome of differentiated cells from NPCs**

Table 1. Assessment of Differentiation of E17 Rat Neural Progenitor cells by Immunocytochemistry

| Culture experiment | % Neu-N       | % GFAP       |
|--------------------|---------------|--------------|
| Day 4 Primary Culture | 18.95 ± 4.78<sup>a</sup> | 17.83 ± 5.34<sup>a</sup> |
| 24h Starvation     | 27.04 ± 3.12<sup>b</sup> | 22.07 ± 3.44<sup>b</sup> |
| 48h Starvation     | 30.26 ± 4.10<sup>b</sup> | 52.41 ± 4.25<sup>b</sup> |

<sup>a,b</sup> percentage of positive cells presented as mean ± SEM; values with different letters within a column indicates significant differences

**Secretome analysis**

Starvation did not only alter heterogeneity of the cells and affect the viability of the cells but also caused secretome changes in conditioned medium from differentiated cells of NPCs. There were 24 proteins (unique peptide≥2 and score sequist HT>0) identified from conditioned medium of differentiated cells of NPCs after 24 hours starvation (CM1) and 21 proteins from cells after 48 hours starvation (CM2) (Figure 4).

There were 15 identified proteins from CM1 and CM2. They were actin, keratin type II cytoskeletal 1, cystatin-C, keratin type II cytoskeletal 6A, peptidyl-prolyl-cis-trans-isomerase A, collagen alpha-1 (I) chain, sparC, statmin, creatine kinase B-type, serum albumin, fructose-bisphosphate aldolase A, 14-3-3 protein zeta/delta, collagen alpha 2 (I) chain, follistatin-related proteins, and collagen alpha 1 (III) chain. Proteins that were identified from CM1 were vimentin, tubulin alpha 1A, tubulin beta III, centrosomal protein of 162 kDa, heat shock cognate 71 kDa, 14-3-3 protein epsilon, fatty acid binding protein epidermal, pyruvate kinase PKM, and ubiquitin 40s ribosomal protein s27a. Six proteins were identified from CM2, superoxide dismutase, fibronectin, prosaposin, insulin-like growth factor binding proteins 2, apolipoproteins E, keratin type I cytoskeletal 10 (Figure 4).

Further analysis using gene ontology analysis (Figure 5) showed that alteration of protein types which were identified in CM1 and CM2 also related to protein function differences in cellular metabolism. Proteins that were identified from CM1 has three major molecular functions of protein binding, catalytic activity, and being structural molecules while proteins in CM2 played roles as molecular function regulator, transducer activity, and protein transporter. According to GO analysis, after 24 hours starvation, secreted proteins involved in biological processes, such as cellular component organization, cellular response, localization whereby a substance or cellular entity like protein complex is transported and or maintained in a specific location within a cell, and biological regulation. Meanwhile, after 48 hours of starvation, the proteins were more emboled in the metabolic process.

Results of the cellular component analysis revealed that proteins which were found in CM1 perform its molecular function in the cell, synapse, organelle, or as a protein complex while proteins in CM2 predominantly act in the extracellular region.

In terms of categorizing into a protein class, identified proteins in CM1 and CM2 can be classified into cell adhesion proteins, chaperones, cytoskeletal proteins, enzyme modulators, extracellular matrix proteins, oxidoreductases, signalling molecules, transfer proteins and transferases. Majority of proteins in CM1 were cytoskeletal and signalling molecules while in CM2 was enzyme modulators. Signaling pathway analysis showed that secreted proteins involved in pathways, such as cadherin, integrin, epidermal growth factor (EGF), fibroblast growth factor (FGF) and Wnt signalling pathways. On the other hand, proteins also were found to be associated in energy production through glycolysis and fructose-galactose metabolism. Besides, identified proteins may also stimulate apoptosis and inflammation mediated by chemokines and cytokines.

**Discussion**

Secretome-based therapy and in-depth study gained much attention since Mirotsou *et al.* reported that the paracrine factor was indeed responsible for beneficial effects than the cells themselves[8]. As reviewed by Vizoso *et al.*, secreteme as a cell-free therapy may resolve safety considerations, can be evaluated for dosage like other pharmaceutical agents, storage can be done without the application of potentially harmful cryopreservative chemicals, more economical, could be modified to desired cell-specific effects and there is possibility for mass production[16].

According to Beer *et al.*, secretomes is the total of factors secreted by cells actively or passively. It can contain soluble proteins such as cytokines, chemokines, or growth factors and also can include lipid, free nucleic acid and extracellular vesicles[17]. Those factors can be obtained from the conditioned medium, the medium where cells are cultured[3]. Mesenchymal stem cells are the common source to produce conditioned medium which contain secreteme for a specific purpose[16-19]. Meanwhile, another potential secreteme source, especially for central nervous system diseases, is neural progenitor cells (NPCs). These cells are extensively investigated for their capacity to signal to the host upon transplantation in experimental neurodegenerative diseases which greatly increase day by day. Indeed, the study on NPCs has risen in the last few decades, but the studies on the differentiated cells have not still been clearly described.
**Figure 4:** Venn diagram and list of identified proteins from Differentiated Cells of E17 Rat Neural Progenitor cells 24h (CM1) and 48h (CM2) after serum deprivation

| CM 1 | CM 1 & CM 2 | CM 2 |
|------|-------------|------|
| Vimentin | Cystatin c | Insulin like growth factor binding protein-2 |
| Tubulin alpha 1A chain | Spare | Prosaposin |
| Tubulin beta-3 chain | Stathmin | Fibronectin |
| 14-3-3 protein epsilon | Collagen alpha-1 (I) chain | Superoxide dismutase |
| Centrosomal protein of 162 kDa | Collagen alpha-1 (III) chain | Apolipoprotein E |
| Pyruvate kinase | Collagen alpha-2 (I) chain | Keratin type 1 cytoskeletal 10 |
| Fatty acid binding protein | 14-3-3 protein zeta/delta | |
| Heat shock cognate 71 kda protein | Serum albumin | |
| Ubiquitin-40S ribosomal protein S27a | Fructose-biphosphatase aldolase A | |
| | Creatine kinase B type | |
| | Follistatin related protein 1 | |
| | Peptidyl-prolyl cis-trans isomerase A | |
| | Actin, cytoplasmic 1 | |
| | Keratin, type II cytoskeletal 1 | |
| | Keratin, type II cytoskeletal 6A | |
Figure 5: Pie chart of gene ontology (GO) analysis of proteins in CM1 (right) and CM2 (left). GO analysis consisted of molecular function (A), biological process (B), cellular component (C), protein class (D), and signaling pathway of the identified proteins (E).
We isolated cells from the whole brain of E17 rat embryos and found that the cells were heterogeneous. Three types of cells were present, which are PSA-NCAM⁺, A2B5⁺, and cells that express both of these markers. Based on our results, after four days of culture, cells that were positive for PSA-NCAM marker was dominant. Polysialylated neural cell adhesion molecules are considered as one of the markers for immature neurons, developing and migrating neurons and neurons of synaptogenesis in the immature vertebrate nervous system. The results showed that the majority of the cells were immature neurons.

Two other types of cells that can be identified are A2B5⁺ and cells that are not only positive for PSA-NCAM but also for A2B5.⁵ Cells that express A2B5⁺ marker are considered to be bipotential glial precursor cells that have a capacity to give rise to oligodendrocytes and type-2 astrocytes in vitro. These cells are also named glial restricted precursor cells (GRPs), which have a characteristic of being positive for A2B5 marker but do not express PSA-NCAM marker.⁶ The last type cells identified by flow cytometry that were found to express both markers, according to Ben-Hur et al. are those cells of oligodendrocytes-type 2 astrocytes (O-2A) lineage which express positive for PSA-NCAM and can be detected in neonatal rat glial cell cultures.⁷

Majority of PSA-NCAM⁺ cells representing immature neurons can be explained related to neurogenesis in the mammalian central nervous system. Neurons are generated primarily in the embryonic period, while most glial cells are generated after birth.⁸ Neurogenesis of mouse or rat cerebral cortex commences around embryonic day 12 (E12), peaks at E15, and finishes around birth. Our findings showed that from E17 rat brains, immature neurons were the predominant cells, but there was also glial cells progenitor that can be obtained showing that gliogenesis at this time was started. In murine, gliogenesis starts at E16 and continues to postnatal life.⁹ According to Bandeira et al., changing numbers of neuronal and non-neuronal cells has an association with developmental stage neurogenesis and correlated with the brain growth.¹⁰

In the process to get secretome from the conditioned medium, cells are being cultured in serum-free conditions. Our study revealed that unfortunately, this step affects the cells themselves and the proteins identified in the conditioned medium.

Starvation of the cells was done by changing the medium with MEM medium, a basal medium which contains limited nutrition and without any addition of serum. During this serum deprivation, we found that the viability of the cells decreased significantly after 48 hours starvation. The decrease in the number of cells viability related to cell death process that can be classified based on morphological appearance into apoptotic, necrotic or autophagic.¹⁰ Young et al. reported that nutrient deprivation could induce neuronal autophagy.¹¹ Autophagy activation is needed to clean misfolded proteins, decrease cytotoxicity, and prevent neurodegeneration.

Mura et al. reported that astrocytes showed a higher capability than neurons to cope with stress.¹² Previous studies proved that this type of glial cell was more able to cope with increased intracellular free Zn²⁺, are capable of counteracting stresses more effectively than neurons due to their antioxidant reserve, express a higher level of antioxidant enzymes than neuronal cells and are less vulnerable to ischemia than neurons.¹³,¹⁴ In line with those studies, we found that neurons decreased in a time-dependent manner while astrocytes increased significantly as an indication that progenitor cells under starvation conditions will tend to differentiate into glial cells, especially astrocytes. Percentage of NeuN positive cells at four days culture, after 24 and 48 hours starvation were 18.95 ± 4.78, 27.04 ± 3.12, and 30.26 ± 4.10, respectively. On the other hand, the percentage of GFAP positive cells were 17.83 ± 5.34, 22.07 ± 3.44, and 52.41 ± 4.25, respectively, as shown in Table 1 and Figure 3.

This phenomenon is caused by the activation of Notch signaling. It prevents neuronal precursors from adopting neuronal fate; instead, it promotes the cells to irreversibly commit to glial fate. In the second step, Notch signaling would inhibit the differentiation of neuron and oligodendrocytes but promote the differentiation into astrocytes.¹⁵ Alterations and starvation also caused changes in the secretome profile that interpreted from protein identification in conditioned medium. During 24 hours of starvation, cells which were dominated by immature neurons, secreted proteins that are used to maintain cell growth, stimulate differentiation, and produce energy, but there were also proteins that were identified to be involved in stress responses and autophagy activation. After 48 hours of starvation, astrocytes that became the dominant cells, secreted proteins that try to keep protecting the remaining neurons. We classified proteins found in CM1 into four groups, which are ‘novel’ extracellular proteins, microtubule organizing proteins, proteins involved in energy metabolism, stress and autophagic activation proteins. Vimentin was earlier known as an intracellular protein but then reported to be also secreted in the extracellular space showing novel protein activity.¹⁶ Shigyo and Tohda reported that extracellular vimentin might be a novel neurotrophic factor that enhances axonal growth activity and motor function recovery after spinal cord injury.¹⁷ Based on GO biological process, it correlated with cellular response to fibroblast growth factor signalling, was found to be involved in axonogenesis and cause positive regulation on glial proliferation. Proteins that involved in energy metabolism detected in CM1; pyruvate kinase, which is a glycolytic enzyme, and fatty acid binding protein that metabolizes lipid-derived sources.¹⁸,¹⁹ Although cells metabolized energy and keep releasing growth factors or proteins that were needed by the cells, under nutrient deprivation misfolded proteins could not be avoided. It urged the cells to activate lysosomal degradation and release stress protein. Ubiquitin and heat shock cognate proteins were two proteins that were found in CM 1 involved in those processes.²⁰,²¹

As the heterogeneity of cells altered after 48 hours starvation, with the dominance of glial and astrocytes cells, proteins that identified only in CM2 also were dominantly secreted by neuroglia cells. They were insulin-like growth factor binding protein, prosaposin, and fibronectin. Astrocytes also secreted a major cholesterol carrier apolipoprotein E.

Insulin-like binding protein will bind to an insulin-like growth factor that acts as neuroprotector to inhibit neuronal damage and enhances neuronal survival largely through the phosphoinositide 3 kinase (PI3k/Akt) signalling pathway.²² Another protein, prosaposin, also has a neuroprotective and glioprotective effect when it binds to the G protein-coupled protein GPR37 and GPR7L1 on their native environment, the astrocytes.²³,²⁴ Besides, astrocytes also secreted fibronectin, one of the extracellular matrix molecules, that dynamically regulates neuronal function such as diffusion of neuroactive substances, receptor activation, and cell adhesion.²⁵ Remaining cells after 48 hours starvation also secreted apolipoprotein E that supports lipid transport and injury repair in the brain.²⁶

These results showed that alteration of cells heterogeneity affected the secretome profile. During a long time of nutrient deprivation, the survival rate of astrocytes and glial cells were higher than neurons. The external environment tends to give rise to different results in the neurogenesis process.²⁷ Nevertheless, astrocytes secreted proteins might still maintain the remaining neurons. These results revealed that astrocytes are not just ‘glue’ for structural support of the neuron but are also responsible for protecting neurons from environmental stress and undesired conditions.
Gene ontology analysis was used to find out whether the differences of identified proteins in CM1 and CM2 affected the role of these proteins in terms of molecular function, biological process, cellular component, protein class, and signalling pathway. Based on the analysis, it was confirmed that not only the type of proteins changed, but also their function and biological process involvement.

Conclusion

Starvation of differentiated cells of NPCs decreased cells viability and caused heterogeneity alterations of the cell population. The cells showed a higher tendency to differentiate to glial cells and those glial cells, especially astrocytes, survived more under nutrient deprivation. The change of cells’ composition in the population of the cells influenced the secretome profile. During 24 hours of starvation, cells secreted proteins that were used to maintain cell growth, stimulate differentiation, and produce energy, but there were also proteins that were identified to be involved in autophagy activation. After 48 hours of starvation, astrocytes became the dominant cells secreting proteins that try to keep protecting the remaining neurons.

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Abbreviations

- NPCs: Neural progenitor cells
- MSCs: Mesenchymal stem cells
- NSC: Neural stem cells
- E17: Embryonic day 17
- HBSS: Hank’s Balanced Salt Solution
- NM: Neurobasal medium
- FBS: Fetal bovine serum
- MEM: Minimum essential medium
- CM: Conditioned medium
- PSA-NCAM: Polysialylated-neural cell adhesion molecule
- PSANCAM-APC: Polysialylated-neural cell adhesion molecule - allophycocyanin
- A2B5-PE: A2B5-phycoerythrin
- GFAP: Glial fibrillary acidic protein
- NeuN: Neuronal nuclei
- HRP: Horseradish peroxidase
- LC/MS-MS: Liquid chromatography/tandem mass spectrometry
- PANTHER: Protein analysis through evolutionary relationships
- GO: Gene ontology
- ANOVA: Analysis of variance
- SEM: Standard error of the mean

Potential Conflicts of Interests

There are no conflicts of interest to report.

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