Медицинская иммунология
2021, Т. 23, № 4,
стр. 677-684

Краткие сообщения
Short communications

Medical Immunology (Russia)/
Meditsinskaya Immunologiya
2021, Vol. 23, № 4, pp. 677-684

ВЛИЯНИЕ РАСТВОРИМЫХ ФАКТОРОВ МАКРОФАГОВ М2-ФЕНОТИПА НА ДИФФЕРЕНЦИРОВКУ КЛЕТОК ЛИНИИ SH-SY5Y

Ращупкин И.М., Шевела Е.Я., Черных Е.Р.

ФГБНУ «Научно-исследовательский институт фундаментальной и клинической иммунологии», г. Новосибирск, Россия

Резюме. Известно, что макрофаги играют важную роль в запуске и регуляции процессов нейрорегенерации. Макрофаги характеризуются выраженной пластичностью, что проявляется в их способности изменять свой фенотип в зависимости от сигналов микроокружения. В условиях патологии одним из индукторов формирования противовоспалительного М2-фенотипа макрофагов является взаимодействие с апоптотическими клетками (эффероцитоз). Ранее нами был разработан оригинальный протокол генерации М2-подобных макрофагов, поляризованных в результате эффероцитоза в условиях депривации сыворотки. Целью настоящего исследования явилась оценка нейрорегенеративного потенциала таких макрофагов (М2 (LS), Low Serum). Исследовали влияние кондиционных сред М2 (LS) на процесс дифференцировки клеток линии SH-SY5Y в сравнении с ретиноевой кислотой (RA). В качестве морфологических критериев дифференцировки оценивали относительное содержание дифференцированных клеток, т.е. клеток с длиной нейритов, превышающей длину тела клетки, а также среднюю длину нейритов на 3-и, 7-е и 13-е сутки. Наряду с этим оценивали соотношение нейроноподобных (N-тип) и эпителиально-подобных (S-тип) клеток в культурах. Клетки SH-SY5Y характеризовались низким уровнем спонтанной дифференцировки как в стандартных условиях (10% FBS), так и при депривации сыворотки (1% FBS). При обработке ретиноевой кислотой клетки SH-SY5Y прекращали делиться и подвергались нейрональной дифференцировке. Культивирование клеток SH-SY5Y в присутствии кондиционной среды (КС)-М2 (LS) (30% v/v) также приводило к значимому возрастанию относительного содержания дифференцированных клеток, средней длины нейритоподобных отростков, а также изменению баланса клеток S- и N-типа в сторону выраженного преобладания последних. При этом выраженность морфологических признаков дифференцировки на ранних этапах дифференцировки (3-и сут.) была достоверно снижена по сравнению с уровнем RA-индуцированных изменений и достигала уровня позитивного контроля только на более поздних стадиях (к 13-м сут.) ($p < 0,05$). В отличие от ретиноевой кислоты, КС-М2 (LS) индуцировали нейрональную дифференцировку клеток SH-SY5Y без подавления их пролиферативной активности. Полученные данные могут свидетельствовать о нейрорегенеративном потенциале М2 (LS) макрофагов

Адрес для переписки:

Ращупкин Иван Михайлович
ФГБНУ «Научно-исследовательский институт фундаментальной и клинической иммунологии»
630099, Россия, г. Новосибирск, ул. Ядринцевская, 14.
Тел.: 8 (383) 236-03-29.
Факс: 8 (383) 222-70-28.
E-mail: iwwwanbets@mail.ru; ct_lab@mail.ru

Address for correspondence:

Rashchupkin Ivan M.
Research Institute of Fundamental and Clinical Immunology
630099, Russian Federation, Novosibirsk,
Yadrintsevskaya str., 14.
Phone: 7 (383) 236-03-29.
Fax: 7 (383) 222-70-28.
E-mail: iwwwanbets@mail.ru; ct_lab@mail.ru

Образец цитирования:

И.М. Ращупкин, Е.Я. Шевела, Е.Р. Черных «Влияние растворимых факторов макрофагов М2-фенотипа на дифференцировку клеток линии SH-SY5Y» // Медицинская иммунология, 2021. Т. 23, № 4. С. 677-684. doi: 10.15789/1563-0625-EOM-2276

For citation:

I.M. Rashchupkin, E.Ya. Shevela, E.R. Chernykh "Effect of M2 macrophage-derived soluble factors on differentiation of SH-SY5Y cells", Medical Immunology (Russia)/ Meditsinskaya Immunologiya, 2021, Vol. 23, no. 4, pp. 677-684. doi: 10.15789/1563-0625-EOM-2276

DOI: 10.15789/1563-0625-EOM-2276

in vitro, который опосредуется через растворимые факторы и проявляется в усилении дифференцировки клеток SH-SY5Y.

Ключевые слова: макрофаги, М2 фенотип, клетки SH-SY5Y, дифференцировка, ретиноевая кислота, нейрорегенерация

EFFECT OF M2 MACROPHAGE-DERIVED SOLUBLE FACTORS ON DIFFERENTIATION OF SH-SY5Y CELLS

Rashchupkin I.M., Shevela E.Ya., Chernykh E.R.

Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation

Abstract. Macrophages play a key role in triggering and regulation of neuroregeneration. The characteristic feature of macrophages is pronounced plasticity, which manifests itself in the ability of macrophages to change their functional phenotype depending on the micromilieu. Apoptotic cell clearance (efferocytosis) is an important inducer of a macrophage polarization to M2 phenotype under pathological settings. Previously, we have developed an original protocol for the generation of M2-like macrophages, polarized by efferocytosis under serum-deprived conditions (M2 (LS), Low Serum). The present study was aimed to assess a neuroregenerative potential of M2 (LS) macrophages. We studied their effect on the differentiation of SH-SY5Y cells in comparison with retinoic acid (RA). As the morphological criteria of differentiation we have assessed the relative content of differentiated cells, i.e., cells with a neurite length exceeding the cell body length, and the average neurite length on days 3, 7, and 13. The ratio of neuron-like (N-type) and epithelial-like (S-type) cells in cultures was also assessed. SH-SY5Y cells were characterized by a low level of spontaneous differentiation, both under standard conditions (10% FBS) and serum deprivation (1% FBS). Upon RA treatment, SH-SY5Y cells stopped proliferating and underwent neuronal differentiation. Cultivation of SH-SY5Y cells in the presence of M2 (LS) conditioned medium also led to a significant increase in the relative content of differentiated cells, the average length of neurite-like processes, as well as a change in the balance of S- and N-type cells towards a pronounced predominance of the latter. The morphological features of differentiation were significantly less pronounced at early stage (day 3) of differentiation as compared with the RA-induced changes and reached the level of positive control only at later stages (day 13) ($p < 0.05$). In contrast to retinoic acid, M2 (LS) conditioned medium induced neuronal differentiation of SH-SY5Y cells without suppressing their proliferative activity. The data obtained may indicate a high neuroregenerative potential of M2 macrophages *in vitro*, which is realized through soluble factors and manifests itself in promoting SH-SY5Y differentiation.

Keywords: macrophages, M2 phenotype, SH-SY5Y cells, differentiation, retinoic acid, neuroregeneration

The work was carried out at the expense of the federal budget for fundamental research on the topic "Justification and development of new technologies for immunomodulation, stimulation of reparative processes and correction of behavioral and addictive disorders based on the use of myeloid, lymphoid and stem cells and/or their secretome products".

Introduction

Macrophages (Ms), being an essential component of innate immunity, play a key role in inflammation, regeneration, and homeostasis maintenance. The main features of Ms are pronounced plasticity and heterogeneity manifested as a potential to change their functional phenotype in response to various microenvironmental signals with formation of many activation states between two opposite "extremities" – classically activated M1 and alternatively activated M2 Ms. A defining M1 characteristic is proinflammatory activity, whereas M2 exhibit anti-inflammatory properties and play a crucial role in stimulating reparative processes and resolution of inflammation [25]. M2 phenotype Ms are induced by a variety of stimuli, such as IL-4/IL-13, immunosupressive cytokines (IL-10,

TGF-β), immune complexes, hormones and vitamins (dexamethasone and vitamin D3). Apoptotic cell clearance (efferocytosis) is an important inducer of M2 macrophage polarization under pathological settings. Previously, we have developed an original protocol for the M2-like Ms generation based on the culture of human blood monocytes under serum-deprived conditions, which contributes to the development of deprivation apoptosis with the subsequent engulfment of apoptotic cells by macrophages.

The Ms obtained in this way are characterized by low antigen-presenting and proinflammatory activities as well as a high level of growth and neurotrophic factors secretion (VEGF, IGF-1, EPO, BDNF, EGF, FGF-basic) [3, 22]. The combination of these properties allows to consider M2 (LS) as a Ms functional phenotype with a high neuroregenerative potential which was confirmed by pilot clinical trials in patients with CNS pathology [4, 23]. *In vitro* studies have revealed one of potential mechanisms for M2 (LS) neuroregenerative effects exerted as the capacity to enhance proliferation and reduce apoptosis of SH-SY5Y neural cells [19]. At the same time, along with increased neural stem cells proliferation, for a successful neuroregeneration and their subsequent differentiation and integration into existing neuronal network were required [24]. However, an influence of M2 (LS) on the neural cell differentiation *in vitro* has not been previously studied.

The present study was aimed to assess the influence of M2-derived soluble factors on the SH-SY5Y neuroblastoma cells differentiation. To this end, we assessed the effects of M2 (LS) conditioned medium on: 1) the number of cells with neurite (s) exceeding cell body lengths; 2) average neurite length in SH-SY5Y cell culture.

Materials and methods

Ms were generated from an adhesive fraction of human peripheral blood mononuclear cells (PBMCs), as described earlier [22]. Briefly, 3-5 × 10^6/ml PBMCs were incubated in RPMI-1640 (Biolot, St. Petersburg, Russia) medium supplemented with 0.05 mM 2-mercaptoethanol, 2 mM sodium pyruvate 0.3 mg/ml L-glutamine, 1% nonessential amino acids, 100 μg/ml gentamycin, 2% autoplasma and 50 ng/ml recombinant human GM-CSF (R&D Systems, USA). After 18-24 hours, the non-adherent cells were removed, and the adhesive cells were cultured for 7 days. The conditioned Ms medium was collected, centrifugated, and stored at -20 °C.

SH-SY5Y cells were cultured in DMEM/F12 (Biolot, St. Petersburg, Russia) medium containing 10% FBS (BioWest, France) (standard conditions) or 1% FBS (conditions of deprivation) at 37 °C in the CO_2 incubator. Cells were passaged after reaching 70-80% monolayer. Cells of passages 3-5 were used for the experiments.

To assess cell differentiation, SH-SY5Y cells were cultured in 6-well plates (4 × 10^5 cells per well) in DMEM/F12 medium with 1% FBS, supplemented with retinoic acid (RA, 10 μM; positive control) or M2 (LS) conditioned medium (30% (v/v), experiment) for 3, 7 and 13 days.

Morphometric analysis was performed using the ImageJ software and the NeuronGrowth plugin [6]. At least 100 cells were analyzed in different fields of view, counting the number of neurite-bearing cells, the length of which exceeded the length of the cell body, and the average neurite length. Three independent experiments were performed. Morphometric analysis was performed at indicated time points (3, 7, and 13 days). For each cell, the length of the primary neurite, defined as a single neurite, or the longest neurite, for cells with more than one neurite, was measured.

Statistical analysis was performed using the STATISTICA software version 8.0 (StatSoft. Inc., USA). The data are presented as median (Me) and interquartile range (IQR, 25-75% quartiles). To reveal a significant difference of the values compared, the Mann–Whitney nonparametric U test was used. A significance level was set at $p < 0.05$.

Results and discussion

Effect of M2 (LS) conditioned medium on the number of differentiated SH-SY5Y cells

Undifferentiated SH-SY5Y cells tend to grow in clumps which are clusters of rounded cells in the center and cells with short processes / neurites along the edges. During the differentiation, the morphology of the culture changes: cells do not form clusters; cell bodies stretch out, acquiring a pyramidal shape with growing neurites; and the processes vs. cell body elongation significantly exceeds. The predominance of the length of the processes over the cell body is one of the main morphological markers of differentiation.

As shown in Table 1, when SH-SY5Y cells were cultured in standard conditions (10% FBS) the relative number of differentiated cells (i.e., cells with neurite length exceeding the length of the cell body) at different stages of differentiation (3, 7, and 13 days) did not outreach 10%, which indicates a low level of spontaneous differentiation. Under deprivation conditions, the number of differentiated cells was decreased almost by 2 times (4.5-5%). In the presence of retinoic acid (positive control), as

TABLE 1. EFFECT OF M2 (LS) CONDITIONED MEDIUM ON MORPHOLOGICAL MARKERS OF DIFFERENTIATION IN THE SH-SY5Y CELL CULTURES

Day	Control (10% FBS)	Deprivation (1% FBS)	RA (10 μM)	M2 (LS) CM (30% (v/v))
	1	2	3	4
Relative amount of differentiated cells, %				
3	9.4	4.5	22.7	15.1
7	5.9	5.0	28.4	13.3
13	5.0	1.0	16.0	36.0
Average length of neurites, μm				
3	24.7 18.6-34.8	16.8 13.5-23.0 2-1: $p_u < 0.005$	37.5 27.8-54.5 3-2: $p_u < 0.005$ 3-1: $p_u < 0.005$	32.1 24.9-43.3 4-3: $p_u < 0.005$ 4-2: $p_u < 0.005$ 4-1: $p_u < 0.005$
7	22.6 15.4-30.9	18.4 13.1-26.1 2-1: $p_u < 0.005$	47.1* 33.5-63.3 3-2: $p_u < 0.005$ 3-1: $p_u < 0.005$	32.4 25.0-44.2 4-3: $p_u < 0.005$ 4-2: $p_u < 0.005$ 4-1: $p_u < 0.005$
13	22.5 17.5-26.4	19.0 11.1-29.7	49.3* 40.5-74.3 3-2: $p_u < 0.005$ 3-1: $p_u < 0.005$	46.209* # 35.6-60.0 4-2: $p_u < 0.005$ 4-1: $p_u < 0.005$

Note. The data are presented as median (Me) and interquartile range (IQR, 25-75% quartiles). *, $p_u < 0.005$ *vs* day 3; #, $p_u < 0.005$ *vs* day 7. CM, conditioned medium.

TABLE 2. EFFECT OF M2 (LS) CONDITIONED MEDIUM ON THE RATIO OF DIFFERENT CELL TYPES IN SH-SY5Y CELLS CULTURES

Type of cells	Day	Spontaneous differentiation		Induced differentiation	
		10% FBS	1% FBS	RA (10 μM)	M2 (LS) CM (30% (v/v))
N-type	3	79	78	89	93
	7	80	81	75	95
	13	63	37	86	98
among them differentiated	3	12.7	6.4	27	17
	7	5	5	28	17
	13	8	3	19	37
S-type	3	21	22	11	7
	7	21	19	25	5
	13	37	63	14	2
N/S-type ratio	3	4:1	4:1	8:1	13:1
	7	4:1	4:1	3:1	19:1
	13	2:1	0.6:1	6:1	49:1

Note. The data of a representative experiment. The relative content of cells was determined by analyzing at least 100 cells. RA, retinoic acid; CM, conditioned medium.

expected, the number of differentiated cells increased significantly, several times higher than a spontaneous differentiation level ($p < 0.05$ as compared with standard and "deprivation" controls). In the presence of M2 (LS) conditioned medium, the percentage of differentiated SH-SY5Y cells also increased. At the early stages of differentiation (3 and 7 days), the number of differentiated cells was lower compared to the positive control. However, by the day 13, the percentage of these cells increased sharply that was more than 2 times higher than in RA-differentiated cultures.

Effect of M2 (LS) conditioned medium on the average length of neurites in SH-SY5Y cultures

The data in Table 1 demonstrated that the average length of neurites in cells, cultivated under standard conditions (10% FBS), was 23-25 μm at all stages of differentiation. Under serum deprivation, the average neurite length significantly decreased down to 17-19 μm. In RA-induced cultures, the average neurite length was increased by 1.5-2 times and 2.2-2.6 times compared with the standard control and the deprivation control, respectively. The increase in neurite length registered on the day 3 continued by the day 7 and further remained at the level reached. Cell cultivation in the presence of M2 (LS) conditioned medium resulted in significantly increased average neurite length vs. standard and deprivation controls. This effect manifested itself on day 3 of culture and persisted up to day 7. At the same time, at the early stages of differentiation, the neurite length in cultures with M2 (LS) conditioned medium was significantly lower than that in the positive control (RA). However, the significantly increased neurite length as high as the level of RA-induced values was observed by the day 13.

Effect of M2 (LS) conditioned medium on the SH-SY5Y cell N/S-type ratio

It is known that the cultures of undifferentiated SH-SY5Y cells contain two morphologically distinct cell types: S-type (adhering to the substrate, epithelial-like cells without processes) and N-type (cells with neurite-like processes). The relative number of each cell type under differentiation conditions is shown in Table 2. The relative number of N- and S-type cells during differentiation of the SH-SY5Y cell line depending on the inducing stimulus is well visualized as the N/S ratio. At the early stages of differentiation, regardless of the serum percentage, the number of N-type cells was 4 times higher than the number of S-type cells. At later stages, a decrease in the number of N-type cells and an increase in S-type cells were observed, which led to a final difference of 2 or more times (2:1 and 0.6:1). RA-induced differentiation was accompanied by an increase in the proportion of N-type cells and a decrease of S-type cells by the days 3 and 13. At the intermediate stage (7 days), the number of S-type cells, on the contrary, increased, which led to a reduction in the N/S ratio from 8:1 to 3:1. The most considerable change in the balance between these cell types was observed in cultures with M2 (LS) conditioned medium: the overwhelming majority of cells was of N-type cells (93, 95, 98%), while the S-type was represented by rare single cells (7, 5, 2%). Interestingly, the majority of N-type cells in these cultures were presented by cells with short processes, not exceeding the length of the cell body (i.e. undifferentiated cells).

The aim of the study was to assess an effect of M2 (LS) Ms on the SH-SY5Y cell differentiation. To address it, we have compared the influence of M2 (LS) conditioned medium and retinoic acid (RA), a well-known and widely used inducer of neuronal differentiation.

The data obtained showed that M2 (LS) conditioned medium, similar to RA, stimulates the differentiation of SH-SY5Y cells exhibited as increased relative content of differentiated cells, the average length of neurite-like processes, as well as a change in the ratio between S- and N-type cells shifted to a pronounced predominance of the latter. At the same time, it is important to note some differences between differentiation processes, induced by retinoic acid and macrophages. Thus, the effect of RA is manifested already at early stage of differentiation (day 3), leading to a significant increase in number of differentiated cells and average neurite length as compared to control cultures. Effect of M2 (LS) conditioned medium is also clearly detected at early stages of differentiation. However, morphological features of differentiation during this period are significantly less pronounced compared to those in RA-induced cultures and reaches the level of positive control only at later stages (by the 13th day). At the same timepoint (day 13) in RA-stimulated cultures a decrease in the relative number of differentiated cells is recorded that may be associated with the long-term presence of retinoic acid as a monostimulus in the culture. Indeed, in many protocols long-term cultivation implies the use of an additional differentiating stimulus (for example, BDNF, IGF-1, laminin coating of plastic, etc) [1].

Some authors have described the presence of two morphologically distinct types of undifferentiated SH-SY5Y cells: S-type (adhering to the substrate, epithelial-like cells without processes) and N-type (cells with neurite-like processes) [21]. Short-term RA treatment (up to 5 days) induces differentiation of N-type cells, while longer-term treatment promotes

proliferation of S-type cells [5]. The authors have also shown that S-type cell proliferation depends on the serum level. These data make possible to explain our results on a twofold increase in S-type cells in RA-induced cultures and a higher proportion of S-type cells in cultures added with 10% FBS.

A distinctive feature of cultures with the addition of M2 (LS) conditioned medium is the preservation of the proliferative activity of SH-SY5Y cells, which is confirmed by the previously obtained data and is consistent with the data obtained by other authors. For instance, Popova et al showed that SH-SY5Y cells continue to proliferate during differentiation [17]. High N-type/S-type cell ratio (N:S, 13:1–49:1), observed in the presence of M2 (LS) conditioned medium and exceeding this index in RA-treated cultures, indicates the predominant proliferation of N-type cells. This provides a considerable number of undifferentiated N-type cells capable of further differentiation. It is reasonable to assume that the differentiation of these certain cells provide a significant enhancement of SH-SY5Y neural differentiation at later stages of differentiation. Thus, soluble factors of M2 (LS) macrophages induce both proliferation and neural differentiation of SH-SY5Y cells. M2 macrophages produce cytokines and growth factors, which activate neurogenesis, oligodendrogenesis and angiogenesis, contributing to the appearance of new cellular elements [2, 26]. By producing trophic and growth factors, M2 Ms provide axonal remodeling, i.e., formation of new axonal collaterals by stimulating the growth of axons, the formation of new synapses and remyelination of axons [8, 14, 20]. We have previously shown that M2 (LS) actively secrete a complex of various neurotrophic, neuroprotective and angiogenic factors, including IGF-1, VEGF, erythropoietin, etc. [3].

It is known that IGF-1, whose production by M2 (LS) macrophages exceeds the level of its production by M1 and M2 macrophages by double-digit rates [3], is able to induce neural differentiation of dental pulp stem cells and stimulate differentiation of SH-SY5Y cells *in vitro* [7, 11]. Moreover, IGF-1 increases the proliferative activity of SH-SY5Y cells [13]. Thus, IGF-1 can significantly contribute to the stimulatory effects of M2 (LS) conditioned medium on proliferation and differentiation of SH-SY5Y cells.

Erythropoietin (EPO) is currently considered as a pleiotropic cytokine which has neurotrophic, neuroprotective, antiapoptotic and mitogenic effects, and plays an important role in the regeneration of various tissues, including nervous tissue [16]. However, EPO influence on SH-SY5Y cell differentiation remains unclear. Pregi et al. showed that EPO inhibits not only apoptosis, but also the differentiation of SH-SY5Y cells induced by staurosporin [18]. In contrast, Yuste et al demonstrated the preservation of the differentiation potential in a similar model [27]. Since M2 (LS) produce a high EPO level, and SH-SY5Y cells express the EPO receptor, it is reasonable to assume that it stimulates SH-SY5Y cells proliferation. At the same time, taking into account the data of Pregi et al, it is impossible to rule out a negative EPO effect on the differentiation of SH-SY5Y manifested as the "delayed" stimulation of the process.

VEGF plays an important role not only in vasculo/angiogenesis, but also in the regulation of neurogenesis, stimulating the proliferation of neuronal precursors either directly [9, 28], or through brain derived neurotrophic factor (BDNF) produced by endothelial cells [12]. Neuroprotective properties of VEGF have been demonstrated both *in vitro* and *in vivo* [10, 15]. The level of VEGF production by M2 (LS) macrophages is significantly higher than that by M1 and M2a (IL-4) [3]. In addition, M2 (LS) also produce BDNF, which promotes differentiation of SH-SY5Y cells and being included into some standard differentiation protocols for these cells [1].

Taken together, the data obtained indicate a high neuroregenerative potential of M2 macrophages *in vitro* revealed by the induced differentiation of SH-SY5Y cells and enabled via soluble factors.

References

1. Agholme L., Lindstrom T., Kagedal K., Marcusson J., Hallbeck L. An *in vitro* model for neuroscience: differentiation of SH-SY5Y cells into cells with morphological and biochemical characteristics of mature neurons. *J. Alzheimers Dis., 2010, Vol. 20, no. 4, pp. 1069-1082.*

2. Butovsky O., Ziv Y., Schwartz A., Landa G., Talpalar A.E., Pluchino S., Martino G., Schwartz M. Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. *Mol. Cell. Neurosci., 2006, Vol. 31, no. 1, pp. 149-160.*

3. Chernykh E.R., Shevela E.Ya., Sakhno L.V., Tikhonova M.A., Petrovsky Ya.L., Ostanin A.A. The generation and properties of human M2-like macrophages: potential candidates for CNS repair? *Cell Ther. Transplant., 2010, Vol. 2, no. 6.* doi: 10.3205/ctt-2010-en-000080.01.

4. Chernykh E.R., Shevela E.Ya., Starostina N.M., Morozov S.A., Davydova M.N., Menyaeva E.V., Ostanin A.A. Safety and therapeutic potential of M2-macrophages in stroke treatment. *Cell Transplant., 2016, Vol. 25, no. 8, pp. 1461-1471.*

5. Encinas M., Iglesias M., Liu Y., Wang H., Muhaisen A., Cena V., Gallego C., Comella J.X. Sequential treatment of SH-SY5Y cells with retinoic acid and brain-derived neurotrophic factor gives rise to fully differentiated, neurotrophic factor-dependent, human neuron-like cells. *J. Neurochem., 2000, Vol. 75, no. 3, pp. 991-1003.*

6. Fanti Z., Martinez-Perez M.E., De-Miguel F.F. NeuronGrowth, a software for automatic quantification of neurite and filopodial dynamics from time-lapse sequences of digital images. *Dev. Neurobiol., 2013, Vol. 71, no. 10, pp. 870-881.*

7. Huang D., Shen S., Cai M., Jin L., Lu J., Xu K., Zhang J., Feng G., Hu Y., Zheng K., Feng X. Role of mTOR complex in IGF-1 induced neural differentiation of DPSCs. *J. Mol. Histol., 2019, Vol. 50, no. 3, pp. 273-283.*

8. Jacobi A., Bareyre F.M. Regulation of axonal remodeling following spinal cord injury. *Neural Regen. Res., 2015, Vol. 10, no. 10, pp. 1555-1557.*

9. Jin K., Zhu Y., Sun Y., Mao X.O., Xie L., Greenberg D.A. Vascular endothelial growth factor (VEGF) stimulates neurogenesis *in vitro* and *in svivo*. *Proc. Natl Acad. Sci. USA, 2002, Vol. 99, no. 18, pp. 11946-11950.*

10. Lange C., Storkebaum E., De Almodóvar C.R., Dewerchin M., Carmeliet P. Vascular endothelial growth factor: a neurovascular target in neurological diseases. *Nat. Rev. Neurol., 2016, Vol. 12, no. 8, pp. 439-454.*

11. Lavenius E., Parrow V., Nanberg E., Pahlman S. Basic FGF and IGF-I promote differentiation of human SH-SY5Y neuroblastoma cells in culture. *Growth Factors, 1994, Vol. 10, no. 1, pp. 29-39.*

12. Louissaint (Jr) A., Rao S., Leventhal C., Goldman S.A. Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain. *Neuron, 2002, Vol. 34, no. 6, pp. 945-960.*

13. Mattsson M.E., Enberg G., Ruusala A.I., Hall K., Pahlman S. Mitogenic response of human SH-SY5Y neuroblastoma cells to insulin-like growth factor I and II is dependent on the stage of differentiation. *J. Cell Biol., 1986, Vol. 102, no. 5., pp. 1949-1954.*

14. Miron V.E., Boyd A., Zhao J.W., Yuen T.J., Ruckh J.M., Shadrach J.L., van Wijngaarden P., Wagers A.J., Williams A., Franklin R.J. M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. *Nat. Neurosci., 2013, Vol. 16, no. 9, pp. 1211-1218.*

15. Nicoletti J.N., Shah S.K., McCloskey D.P., Goodman J.H., Elkady A., Atassi H., Hylton D., Rudge J.S., Scharfman H.E., Croll S.D. Vascular endothelial growth factor is up-regulated after status epilepticus and protects against seizure-induced neuronal loss in hippocampus. *Neuroscience, 2008, Vol. 151, no. 1, pp. 232-241.*

16. Peng B., Kong G., Yang C., Ming Y. Erythropoietin and its derivatives: from tissue protection to immune regulation. *Cell Death Dis., 2020, Vol. 11, no. 2, 79.* doi: 10.1038/s41419-020-2276-8.

17. Popova D., Karlsson J., Jacobsson S.O.P. Comparison of neurons derived from mouse P19, rat PC12 and human SH-SY5Y cells in the assessment of chemical- and toxin-induced neurotoxicity. *BMC Pharmacol Toxicol., 2017, Vol. 18, no.1, p. 42.*

18. Pregi N., Vittori D., Pérez G., Pérez Leirós C., Nesse A. Effect of erythropoietin on staurosporine-induced apoptosis and differentiation of SH-SY5Y neuroblastoma cells. *Biochim. Biophys. Acta, 2006, Vol. 1763, no. 2, pp. 238-246.*

19. Rashchupkin I.M., Maksimova A.A., Sakhno L.V., Ostanin A.A., Shevela E.Ya., Chernykh E.R. The effect of M2 macrophage-derived soluble factors on proliferation and apoptosis of SH-SY5Y cells. *Bulletin of Experimental Biology and Medicine, 2021, Vol. 171, no. 1, pp. 59-63.* (In Russ.)

20. Rawji K.S., Mishra M.K., Yong V.W. Regenerative capacity of macrophages for remyelination. *Front Cell Dev. Biol., 2016, Vol. 4, 47.* doi:10.3389/fcell.2016.00047.

21. Ross R.A., Spengler B.A., Biedler J.L. Coordinate morphological and biochemical interconversion of human neuroblastoma cells. *J. Natl Cancer Inst., 1983, Vol. 71, no. 4, pp. 741-747.*

22. Sakhno L.V., Shevela E.Y., Tikhonova M.A., Ostanin A.A., Chernykh E.R. The phenotypic and functional features of human M2 macrophages generated under low serum conditions. *Scand. J. Immunol., 2016, Vol. 83, no. 2, pp. 151-159.*

23. Shevela E., Davydova M., Starostina N., Yankovskaya A., Ostanin A., Chernykh E. Intranasal delivery of M2 macrophage-derived soluble products reduces neuropsychological deficit in patients with cerebrovascular disease: a Pilot Study. *J. Neurorestoratol., 2019, Vol. 7, no. 2, pp. 89-100.*

24. Ulrich H., Resende R. Mechanisms of neural differentiation and integration. *Semin. Cell Dev. Biol., 2019, Vol. 93, pp. 1-3.*

25. Xiang X., Li Y., Fu M., Xin H-B. Polarizing macrophages *in vitro*. *Methods Mol. Biol., 2018, Vol. 1784, pp. 119-126.*

26. Xiong X.Y., Liu L., Yang Q.W. Functions and mechanisms of microglia/macrophages in neuroinflammation and neurogenesis after stroke. *Prog. Neurobiol., 2016, Vol. 142, pp. 23-44.*

27. Yuste V., Sánchez-López I., Solé C., Encinas M., Bayascas J., Boix J., Comella J. The prevention of the staurosporine-induced apoptosis by Bcl-xL, but not by Bcl-2 or caspase inhibitors, allows the extensive differentiation of human neuroblastoma cells. *J. Neurochem., 2002, Vol. 80, no. 1, pp. 126-139.*

28. Zhu Y., Jin K., Mao X.O., Greenberg D.A. Vascular endothelial growth factor promotes proliferation of cortical neuron precursors by regulating E2F expression. *FASEB J., 2003, Vol. 17, no. 2, pp. 186-193.*

Авторы:

Ращупкин И.М. *— аспирант лаборатории клеточной иммунотерапии ФГБНУ «Научно-исследовательский институт фундаментальной и клинической иммунологии», г. Новосибирск, Россия*

Шевела Е.Я. *— д.м.н., ведущий научный сотрудник лаборатории клеточной иммунотерапии ФГБНУ «Научно-исследовательский институт фундаментальной и клинической иммунологии», г. Новосибирск, Россия*

Черных Е.Р. *— д.м.н., профессор, член-корр. РАН, заведующая лабораторией клеточной иммунотерапии ФГБНУ «Научно-исследовательский институт фундаментальной и клинической иммунологии», г. Новосибирск, Россия*

Authors:

Rashchupkin I.M., *Postgraduate Student, Laboratory of Cellular Immunotherapy, Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation*

Shevela E.Ya., *PhD, MD (Medicine), Leading Research Associate, Laboratory of Cellular Immunotherapy, Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation*

Chernykh E.R., *PhD, MD (Medicine), Professor, Corresponding Member, Russian Academy of Sciences, Head, Laboratory of Cellular Immunotherapy, IResearch Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation*

Поступила 14.03.2021
Отправлена на доработку 11.05.2021
Принята к печати 19.05.2021

Received 14.03.2021
Revision received 11.05.2021
Accepted 19.05.2021